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CHANGES IN CIRCULATING EOSINOPHILS IN JUVENILE DIABETICS IN RESPONSE TO EPINEPHRINE, ACTH AND HYPOGLYCEMIA*

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THE alteration of carbohydrate metabolism in Cushing's syndrome and during ACTH (1) and cortisone (2) administration has led to the belief that, at least in some cases of diabetes mellitus, an elevated level of adrenal cortical function may be of etiologic significance. On the other hand, recent studies of the response of diabetics to stress (3) and determinations of urinary steroid excretion in diabetics (4, 5) have led some to the belief that perhaps there exists a degree of decreased adrenal cortical function.

It has been demonstrated repeatedly that a variety of stress situations and the administration of ACTH and epinephrine all induce a pronounced fall in the level of circulating eosinophils, due presumably (except possibly for epinephrine) to stimulation of the adrenal cortex (6–11). This fact, together with the ease of counting eosinophils, has led to the introduction of the ACTH test (12) and the epinephrine test (13), both of which, within certain limits, are valuable tools for the estimation of adrenocortical function.

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In earlier work (14) at this camp, results were obtained which suggested low-normal or subnormal responses to epinephrine and ACTH as measured by the eosinophil fall in juvenile diabetics.

The present study was undertaken in an attempt to clarify further the factors involved. It should be emphasized that, although these studies were made with the evaluation of adrenocortical response and reserve in mind, they represent also studies of variations in the level of circulating eosinophils in juvenile diabetics under certain circumstances. The accumulation of such data in various conditions will eventually shed some light on the nature of the factors which influence this important and simple measurement.

MATERIALS AND METHODS

All diabetic subjects were campers at the Elliott P. Joslin Camp in Charlton, Massachusetts. Strict medical supervision notwithstanding, they were living under conditions very similar to those of any nondiabetic camp. Each of the subjects had well controlled diabetes and none had ketone bodies in the urine. The routine laboratory examinations which facilitated this control included the following: four daily qualitative Benedict tests, determination of 24-hour urinary sugar excretion, daily tests for acetone, and eight blood sugar determinations per week. All subjects had a routine physical examination and, with 3 exceptions, had no disease other than diabetes. The exceptions were 2 boys with epilepsy and 1 with Addison's disease.

Because of the now well known diurnal variations in the level of circulating eosinophils, which must perforce be related to daily activities and strains, the inclusion of a control group as similar as possible to the examined juvenile diabetics was deemed essential. This control group was made up of campers from the Springfield Boys' Club Camp in Brimfield, Massachusetts. These boys were living under conditions similar to those at the Joslin Camp and all of them were considered to be in good health.

The 59 diabetic subjects had an average age of 11.2 years. They volunteered participation in a total of eighty-six epinephrine tests, twelve ACTH tests and eight control tests with salt solution (saline test). The 30 boys in the control group had an average age of 12.6 years, and each participated in one epinephrine test. Ten additional nondiabetics in the same age group were subjects for saline tests. All tests were performed in the same way and under identical circumstances. Capillary blood, which was used for both blood sugar and eosinophil determinations, was obtained from the ear lobe. Blood sugar concentration was determined by the method of Folin and Malmros (15). Eosinophils were counted by Randolph's phloxine propylene glycol method (16). Blood was drawn to the 0.1 mark in at

least two standard leukocyte pipettes after the first drop was removed from the skin. Randolph's solution was drawn to the 1.1 mark and the pipettes shaken vigorously. After allowing twenty minutes to elapse to insure maximal staining, the pipettes were shaken again for two and a half minutes and, after checking for clots, used to fill two Levy counting chambers of 0.2 mm. depth. The average count was multiplied by 3.12 to express the result in eosinophils per cubic millimeter of blood.

Subjects weighing under 100 pounds received 0.25 cc. of 1:1000 epinephrine (Parke Davis and Co.) subcutaneously in the deltoid region directly after blood samples for the initial determinations were taken. Subjects weighing more than 100 pounds received 0.30 cc. Exactly four hours later, capillary blood was again taken for blood sugar and eosinophil determinations. The change in the level of circulating eosinophils was expressed as the percentile drop from the initial count. The saline tests were conducted in exactly the same way, the only difference being that 0.85 per cent solution of sodium chloride was injected instead of epinephrine. For the ACTH tests, the subjects received 25 mg. of ACTH (Armour) intramuscularly. The procedures were identical with those for the epinephrine and saline tests except that at least four pipettes were filled and the average count for four chambers was used. In the studies on insulin hypoglycemia, at least two pipettes and two chambers were used; the first capillary blood samples were taken at the time of the hypoglycemia before treatment, and again four hours later.

RESULTS

Epinephrine tests in nondiabetics

Thirty nondiabetic boys each had one epinephrine test carried out at the same time in the morning as for the diabetic boys. Breakfast was held at the same time in both camps and the morning programs of the two camps were similar. The results showed falls in the levels of circulating eosinophils which ranged from 42 to 86 per cent, with a mean of 64 per cent and a standard deviation of 11 per cent (Fig. 1). Of the 30 boys, 27 showed a fall of 50 per cent or more. The mean basal eosinophil count before epinephrine administration was 327 per cu. mm. Blood sugar values ranged between 87 and 112 mg. per 100 cc. These results are similar to those found in a series of 12 adults by Recant *et al.* (13). These adults responded to 0.3 cc. of epinephrine with an eosinophil drop which ranged from 49 to 80 per cent, with a mean of 62 per cent. Ten saline tests were given to 10 nondiabetic boys at the same time and under similar circumstances. The mean response was a 4 per cent drop in the level of circulating eosinophils; the responses ranged from a drop of 35 per cent to a rise of 15 per cent.

Epinephrine tests in diabetics

Morning tests. Single epinephrine tests were made in 25 diabetic boys at 7:15 A.M., after an overnight ten-hour fast. The mean response was a 36 per cent drop in the eosinophil level (Fig. 1); the responses ranged from a drop of 81 per cent to a rise of 19 per cent and the standard deviation

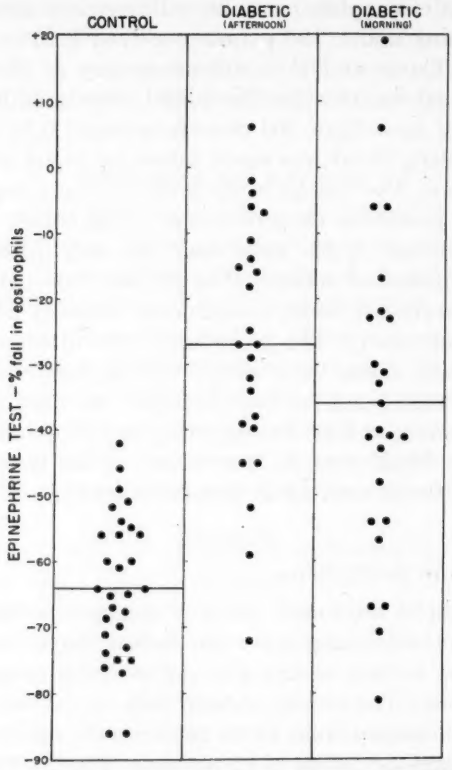

FIG. 1. Fall in circulating eosinophils in normal and in diabetic boys following epinephrine.

was 24 per cent. Only 7 of the 25 boys showed a fall of 50 per cent or more. The mean basal eosinophil count was 252 per cu. mm.

Afternoon tests. In a previous paper from this camp (14) evidence was presented which indicated that those diabetics who had low blood sugar values at the time of the epinephrine test tended to give the lowest responses. Since with very few exceptions all of the boys were receiving NPH insulin and since with this insulin the blood sugar tended to be rela-

tively high in the morning and maximally depressed between 4:00 and 5:00 P.M., it was decided to conduct a series of tests at 4:45 in the afternoon so that a comparison of these results could be made with those of the morning tests. Twenty-four boys participated in the afternoon tests and in a number of them the test was repeated once or twice. The mean response for the total of 42 tests was a fall of 26 per cent. In the statistical comparison with other groups (shown in Figure 1) the data used are only those of the first test given to each of the 24 subjects. There was a mean fall of 27 per cent, ranging from a drop of 72 per cent to a rise of 6 per cent, with a standard deviation of 19 per cent. Only 3 of the 24 subjects showed a fall of 50 per cent or more. The mean eosinophil count at the time of the test was 276 per cu. mm. Saline tests were conducted in 8 diabetic boys. The mean response was a drop of 3 per cent, and the responses ranged from a drop of 17 per cent to a rise of 12 per cent.

Statistical analysis of these data by means of the "t" test for independent groups reveals the difference in response between the control (nondiabetic) group and both the morning and the afternoon diabetic groups to be significant at the $P < .01$ level. The difference in response between the morning and the afternoon groups, however, was found not to be statistically significant. Although there was a tendency toward smaller eosinophil responses when the pre-epinephrine blood sugar level was lowest, this effect could not be shown statistically.

Epinephrine test in relation to pre-epinephrine blood sugar level

It was thought that the tendency toward a lower response when the pre-epinephrine blood sugar level was low might be due to a prolonged period of asymptomatic hypoglycemia preceding the test. This prolonged hypoglycemia might stimulate the hypophyseal-adrenal cortical axis to some degree of activity above basal requirements, so that at the time of the epinephrine test there might exist a degree of reduced cortical responsiveness. To test this possibility, five blood sugar determinations were made on each of 16 boys who were given the epinephrine test in the afternoon at the same time and under the same circumstances as the other afternoon epinephrine tests. In this group, however, the daily insulin dosage was manipulated and supplementary carbohydrate feedings given, so that the desired blood sugar levels could be approximated. Most of the participants in this study were new arrivals at camp who had not experienced insulin reactions for months preceding the date of their arrival. The other subjects were boys who had not had a hypoglycemic reaction for at least three days preceding the day of the test. This precaution was felt necessary to rule out any possible long-standing effect of insulin hypoglycemia on adrenal cortical response.

Blood sugar determinations were made at 7:20 A.M., 11:15 A.M., 4:45 P.M., 7:45 P.M. and 8:45 P.M. The results of these determinations are plotted in Figure 2. It must be remembered that the lines in the figure do not by any means represent the subject's continuous blood sugar level over the course of the day, since meals were taken at the usual times; they merely indicate trends in the blood sugar.

Shown in Part A of Figure 2 are the results obtained in 6 boys whose blood sugar levels were generally low for some hours preceding the test. The responses to the epinephrine test were falls in eosinophils which ranged from 6 to 52 per cent, with a mean of 33 per cent. In Part B are plotted the results obtained when the blood sugar levels of 6 of the subjects were kept, for the most part, somewhat elevated. The responses here varied from a rise of 8 per cent to a fall of 45 per cent; the mean response was a drop of 27 per cent.

Comparison of these two groups showed that in both there was just as much variety of response as in either one of the two main groups, and that mean responses did not differ significantly from each other or from the means obtained from the morning and afternoon epinephrine tests. Thus it would appear that as long as the blood sugar remains at an asymptomatic level, whether it be high or low for some time preceding the injection of epinephrine will make no difference in the outcome of the test. Furthermore, these studies indicate that the general subnormal response to the epinephrine test on the part of the diabetic is not related, at least directly, to the blood sugar level.

The next question considered was whether or not this tendency toward a lower response when the pre-epinephrine blood sugar was low could be related to a more or less acute drop to this pre-epinephrine level from a previous higher level. The results in the 4 boys who participated in this part of the study are shown in Part C of Figure 2. The mean response was a fall of 73 per cent and all drops were greater than 50 per cent (up to 95 per cent). These findings were contrary to what we had expected; none of the subjects manifested symptoms of hypoglycemia at the time of the test. However, as may be seen in Figure 2, in each case the blood sugar fell from an initial level of almost 200 mg. per 100 cc. to a value which ranged from 57 to 62 mg. per 100 cc. It is possible that at the time the epinephrine was given to these subjects, they were in a state of asymptomatic insulin hypoglycemia and that the unusually large responses were due to the additive effects of the epinephrine and the stress of the insulin hypoglycemia.

ACTH tests in 12 diabetics, including 1 with Addison's disease

One ACTH test was made in each of 12 subjects who had previously

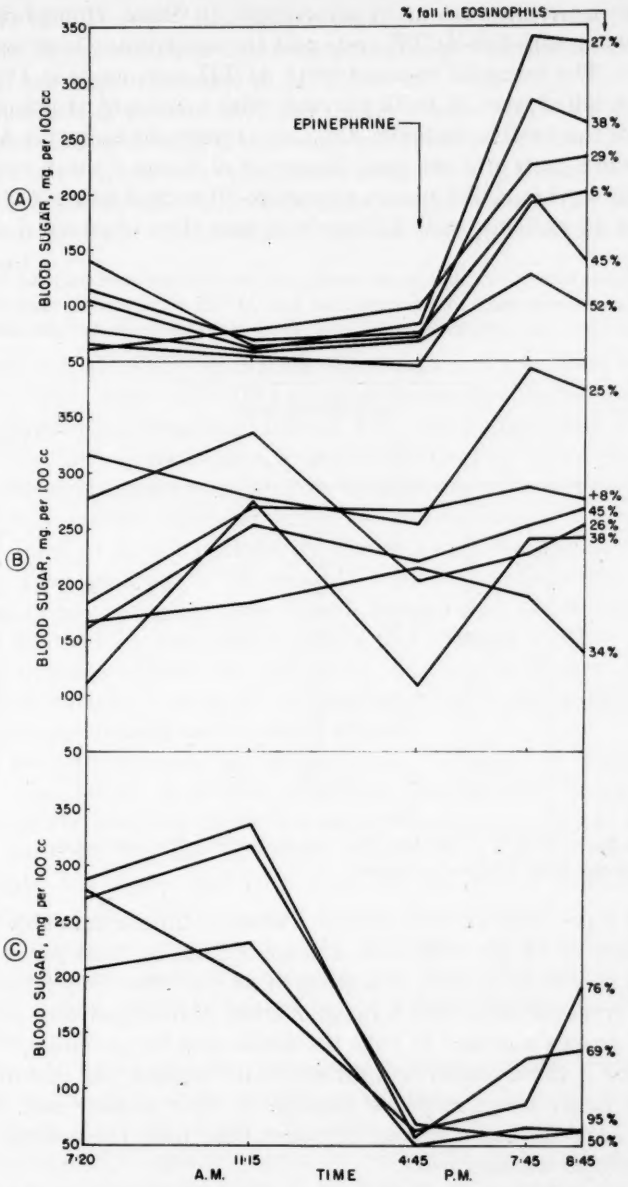

FIG. 2. Responses to epinephrine tests in: (A) 6 diabetics in whom the blood sugar level was low prior to the test; (B) 6 diabetics in whom the blood sugar level was relatively high preceding the test; and (C) 4 diabetics in whom the blood sugar level fell rather acutely during the time preceding the test.

responded poorly to two, or in some cases to three, epinephrine tests. The results of both the ACTH tests and the epinephrine tests are shown in Table 1. The eosinphil response to 11 ACTH tests made in 11 diabetic boys was a fall of from 23 to 77 per cent, with a mean of 54 per cent. The response of the twelfth diabetic, A.C., an 11-year-old boy with Addison's disease, was a drop of 4 per cent. Recant *et al.* found a mean response of 76 per cent to the ACTH test in a series of 20 normal adults (13), and in a series of 43 patients with Addison's disease they obtained a mean re-

TABLE 1. COMPARISON OF CHANGES IN LEVEL OF CIRCULATING EOSINOPHILS FOLLOWING THE ADMINISTRATION OF EPINEPHRINE AND ACTH TO JUVENILE DIABETICS

Subject	Per cent fall* in eosinophils			
	Epinephrine tests			ACTH test
	I	II	III	
A	17	34		52
B	40	25		47
C	6	38		64
D	27	22	13	57
E	18	28	+19	58
F	15	27		46
G	11	15		41
H	26	+8		23
I	7	13		77
J	3	15		65
K	25	2		58
X**	34	17		4

* With the exception of the two rises, as denoted by the plus signs.
** A diabetic with Addison's disease.

sponse of 8 per cent. Wilson *et al.* in a series of 25 normal subjects found a mean drop of 74 per cent, with a range of from 54 to 94 per cent, in response to 25 mg. of ACTH; in a group of 25 diabetics they found a mean response of 65 per cent, with a range of from 26 to 90 per cent (17). Since the same dosage was used in both the adults and the juveniles, one would expect that if there should be a difference in response, the juveniles would have the larger mean response because of their smaller size. That the juvenile diabetics had a smaller response than both the normal and diabetic adults seems significant.

As may be seen in Table 1, there is no relationship between the responses to epinephrine and those to ACTH. Subject I had a perfectly normal response to ACTH (77 per cent), whereas his responses to two epinephrine

tests were falls of only 7 and 13 per cent. On the other hand, Subject B had 40 and 25 per cent decreases in response to epinephrine, and a decrease of only 47 per cent in response to ACTH.

It is interesting to note that the diabetic with Addison's disease, although responding to ACTH with only a 4 per cent fall, had decreases of 17 and 34 per cent in two epinephrine tests. This is consistent with the findings of Recant *et al.* (13), who made epinephrine tests in a series of 11 nondiabetic patients with Addison's disease, and observed a mean fall of 21 per cent.

Additional studies in a diabetic with Addison's disease

Determinations of the circulating eosinophils and the blood sugar concentration were made at 11:00 A.M. on as many days as possible without interfering with the camp activities of A.C., the diabetic boy with Addison's disease. He was receiving a maintenance dose of 6.25 mg. of cortisone twice a day at the same time, and on the first day of each month 2 cc. of desoxycorticosterone trimethylacetate[1] intramuscularly. The eosinophil levels are plotted as one ordinate in Figure 3 and the insulin dosage required to keep the subject "in control" is plotted as the other ordinate, with a common abscissa of time. It will be seen that the eosinophil level rose to a peak which was reached on the day (August 1) when the intramuscular desoxycorticosterone was given. The insulin dosage curve is in part almost the mirror image of the eosinophil curve; the eosinophil count and insulin requirement are inversely related.

If the period preceding the intramuscular injection of desoxycorticosterone is considered an interim of relative insufficiency, because of the depletion of the previous month's dosage, then we are at a loss to explain on that basis the increase in insulin sensitivity which occurred at this time. Köhler has shown that DCA increases the glucose tolerance of diabetic patients (18), and Cheng and Sayers have reported that prolonged DCA administration induces a state of insulin hypersensitivity (19). Further studies are necessary to insure that both the insulin dosage and eosinophil curves are truly related to the administration of desoxycorticosterone.

During A.C.'s stay at camp, symptoms of hypoglycemia developed four or five times. On three such occasions it was possible to study the effect of this stress on the level of his circulating eosinophils. As is described in detail subsequently, capillary blood for eosinophil and blood sugar determinations was taken at the time of the onset of the apparent insulin reac-

[1] A Ciba preparation, kindly furnished by Dr. George W. Thorn and Dr. Dalton Jenkins of the Peter Bent Brigham Hospital, Boston.

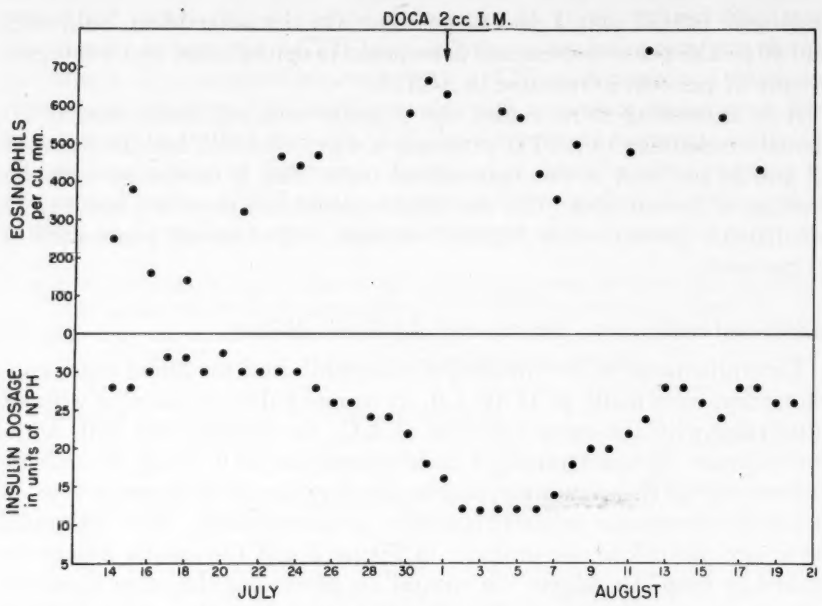

FIG. 3. Eosinophil counts and insulin dosage over a five-week period in a juvenile diabetic with Addison's disease.

tion, and then four hours later. However, in the case of A.C., it was not possible to carry out two of the blood determinations. During one such supposed hypoglycemic reaction (with, however, a pre-treatment blood sugar value of 111 mg. per cent) A.C. responded with an eosinophil drop of 25 per cent; on the other two occasions his eosinophils dropped 25 per cent and 42 per cent. As has been previously stated, his eosinophil responses to two epinephrine tests were decreases of 17 and 34 per cent and his response to an ACTH test was a decrease of 4 per cent. These data suggest the possibility that ACTH release may not be entirely responsible for the cortical response to either epinephrine or hypoglycemia. This is in accord with the experience of Thorn *et al.* (20) in cortisone-treated patients with Addison's disease.

Eosinophil response to severe hypoglycemia

The effect of severe hypoglycemia on the level of circulating eosinophils was studied in 14 boys who experienced a total of 18 insulin reactions of varying degrees of severity. All reactions were spontaneous and of short duration; all were treated within five or ten minutes of the onset of symptoms. A capillary blood sample for eosinophil count and blood sugar determination was taken first and then the subject was immediately treated

with 100 to 200 cc. of ginger ale if he could be treated orally, or 20 cc. of 50 per cent glucose intravenously if he had to be treated parenterally. Four hours from the time of onset of the insulin reaction, blood was again taken for eosinophil and sugar determinations.

The mean response was a fall of 46 per cent in the level of circulating eosinophils; the responses ranged from a rise of 13 per cent to a fall of 94 per cent. The pre-treatment blood sugar values ranged from 35 to 106 mg. per 100 cc., with a mean of 57 mg. per 100 cc. As may be seen in Table 2, there is no consistent relationship between blood sugar level and the extent of the eosinophil drop. The evidence obtained here is not consistent with the observation of Perlmutter and Mufson that an eosinophil fall occurs only when the true blood sugar falls below 35 mg. per 100 cc. (21); nor is it in agreement with the conclusions of Gershberg and Long who, using the decrease in rat ascorbic acid as an index of adrenal cortical activity, found a response only when the blood sugar level fell below 40 mg. per 100 cc. (22).

Even when account is taken of the difference in methods of blood sugar

TABLE 2. COMPARISON OF CHANGES IN LEVEL OF CIRCULATING EOSINOPHILS IN RESPONSE TO HYPOGLYCEMIA AND TO EPINEPHRINE

Subjects C, D and F were observed during more than one insulin reaction

Subject	Pre-treatment blood sugar level (mg./100 cc.)	Per cent fall* in eosinophils in response to hypoglycemia	Per cent fall* in eosinophils in response to epinephrine
A	38	46	
B	70	20	41
C	50	43	
C′	62	60	
D	106	86	11, 15
D′	70	31	
E	54	62	18, 28, +19
F	35	47	15, 27
F′	63	94	
F″	40	26	
G	61	61	4, 37
H	81	90	27, 13, 22
I	39	1	51
J	45	67	
K	59	55	57
L	43	40	32, 10, 28
M	67	+13	39, 15
N	40	8	27

* With the exception of the rise denoted by a plus sign.

determination employed in this study and in those just referred to, there are some subjects whose blood sugar levels did not drop below 40 mg. per 100 cc., and all subjects were obviously in a state of insulin reaction. Probably also to be considered in addition to the absolute blood sugar level is the rate at which the blood sugar fell from a previous high level, as it concerns both the insulin reaction itself and the adrenal cortical response to it. Two subjects with pre-treatment blood sugar concentrations of 106 and 81 mg. per 100 cc. had drops in eosinophil levels of 86 and 90 per cent, respectively. On the other hand, 2 boys with pre-treatment blood sugar values of 39 and 40 mg. per 100 cc. had eosinophil drops of only 1 and 8 per cent.

It is interesting to compare the eosinophil response to an insulin reaction with that to an epinephrine test made several days before or after a hypoglycemic episode, during conditions of relative normoglycemia. These responses are listed in Table 2. Subject E may be seen to have responded to hypoglycemia with a 62 per cent reduction in his eosinophils, whereas his responses to three epinephrine tests were drops of 18 and 28 per cent and a rise of 19 per cent. This pattern of response is seen in about half of the subjects who underwent epinephrine tests. In the other half, the pattern approached the type of response given by Subject I, who showed a 1 per cent fall in response to an insulin reaction and a 51 per cent fall after epinephrine.

These results indicate that the eosinophil response to hypoglycemia is to a large degree independent of the response to epinephrine. It is possible that the response to hypoglycemia may not be entirely mediated by the mechanism of epinephrine release. That there may be an effect of hypoglycemia on the pituitary-adrenal system without epinephrine mediation is evidenced by the work of Gelhorn and Frank who showed adrenal cortical activity in response to hypoglycemia in demedullated rats (23).

DISCUSSION

If the epinephrine test can be considered to be a valid test for pituitary-adrenal cortical responsiveness, then the data presented here suggest strongly that most of the juvenile diabetics studied exhibited a subnormal response. However, the epinephrine test has recently suffered a good deal of criticism. Among others, Jeffries *et al.* have pointed out that a low response to this test does not always indicate insufficiency, as measured by response to ACTH and by 17-ketosteroid and potassium excretion (24). Fisher and Fisher reported that of the subjects they studied, 23 per cent showed diurnal drops of 40 per cent or more (25). An added criticism as far as diabetics are concerned derives from the work of Kjems, who showed that the eosinophil levels in diabetics who were being treated with insulin

were considerably more fluctuant than those of nondiabetics and diabetics treated without insulin (26). Our own studies have shown that an individual may show different responses to two or three epinephrine tests given under comparable conditions. It is evident that a single epinephrine test cannot be considered as a reliable indication of pituitary-adrenal cortical responsiveness. However, the epinephrine test seems of value as a tool in group studies.

Last and Pitesky showed that the repetitive administration of epinephrine to dogs was followed by a progressively diminished eosinophil response which was not the result of adrenal exhaustion or depletion of endogenous ACTH but was believed to be due to an adaptation of the organism to epinephrine stress, with decreased tissue utilization of corticoids (27). The possibility that juvenile diabetics, who frequently experience hypoglycemia with consequent release of endogenous epinephrine, could show similar adaptation must be considered. We cannot exclude this possibility, but as a complete explanation of the subnormal response of the diabetic group it seems unlikely, in view of the fact that a large proportion of the subjects studied had not experienced frequent or severe insulin reactions. The low responses to the ACTH tests support the same view and also suggest that the block lies in the adrenals or beyond the adrenals, perhaps in secondary and tertiary factors involved in the eosinopenia induced by cortical steroids. In the absence of any data suggesting that adrenal cortical extract does not show the same direct, presumably lytic (28) effect upon the circulating eosinophils in the diabetic as it does in normal subjects and in patients with Addison's disease (13), we must accept as the most cogent explanation for the low response in diabetics to the ACTH test as well as to the epinephrine test, that of adrenal cortical unresponsiveness—which by some workers has been assumed to be synonymous with adrenal insufficiency. This interpretation as regards the epinephrine test is, however, still open to question, because the mechanism by which epinephrine induces an eosinopenia is not fully understood (29). Whether this unresponsiveness is a concomitant of diabetes or whether it develops during the course of the disease, perhaps as a result of the manifold stresses imposed upon the homeostatic mechanism, is a matter of speculation at the present time. We have been unable to find any correlation between the duration of the disease and the degree of unresponsiveness. Field and Marble, however, in their study of adrenal response to surgical stress in diabetics (3), reported a suggestive correlation between the degree of adrenal sluggishness as measured by eosinophil falls and the duration of diabetes.

The relation between the blood sugar level and the number of circulating white blood cells, mainly the lymphocytes and the eosinophils, has been

the object of many studies. Insulin hypoglycemia has been shown to induce a fall in the circulating lymphocytes and eosinophils in nondiabetics as well as in diabetics (8, 21). Others have shown in rats (30), dogs (31) and in human diabetics and nondiabetics (32, 33, 34) that a rapidly rising blood sugar level induces an eosinopenia. This suggests that rapid shifts in blood sugar concentration may constitute a physiologic stress. It is conceivable that in the diabetic whose blood sugar level is frequently subject to large and rapid changes, the maintenance of a high level of adrenal cortical function may ultimately lead to a state of insufficiency.

SUMMARY AND CONCLUSIONS

The mean fall in circulating eosinophils following epinephrine in controlled juvenile diabetics was significantly less than that obtained in nondiabetics. In the diabetics the epinephrine tests were made both in the forenoon when the blood sugar level was relatively high and in the afternoon when it was generally lowest. There was no significant difference in the responses obtained at these two times.

It is suggested that provided there is a fairly static level, the blood sugar concentration, whether high or low at the time of and preceding the test, will not directly influence the eosinophil response to epinephrine.

The mean response to the ACTH test in diabetics was shown to be significantly lower than the generally accepted response in nondiabetics.

Observations of the eosinophil count and insulin dosage in a patient with diabetes and Addison's disease are reported.

Insulin hypoglycemia induced an eosinophil response which could not be correlated with the blood sugar level and the response to the epinephrine test made under conditions of relative normoglycemia.

It is concluded that the low mean response to the epinephrine test and to the ACTH test in juvenile diabetics may be explained on the basis of adrenal cortical unresponsiveness, which has been assumed by some workers to be synonymous with adrenal cortical insufficiency. It is suggested that rapid changes in blood sugar concentration may constitute a stress, necessitating a high level of adrenal cortical function, which may ultimately lead to a state of relative adrenal insufficiency.

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OBSERVATIONS ON THE ANTIDIURETIC SUBSTANCE IN HUMAN SERUM

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LLOYD and Lobotsky (1) reported that human serum contains an antidiuretic substance which can be detected by the method of assay developed by Birnie *et al.* (2), the serum being injected intraperitoneally into hydrated rats. They stated that "the activity of 1 cc. of human serum was usually of the order of that of 5 milliunits or less of Pitressin." They gave no definite information about the antidiuretic titer found in individual cases, as they were concerned with the possible role of the ratio of this titer to the urinary concentration of corticosteroids in controlling water diuresis. However, examination of their figures shows that the majority of the sera tested did show considerable antidiuretic activity in rats. In particular, the greatest activity in normal adults was found following twelve hours' dehydration. Considerable activity was also found in the sera of patients with adrenal deficiency.

Slessor (3), using the same technique, also showed that the serum from normal subjects was antidiuretic after fourteen hours' dehydration, and gave evidence of an even higher titer in the serum of patients with Addison's disease. Birnie *et al.* (2) were able to demonstrate an antidiuretic substance in the serum of normal and adrenalectomized rats. Dicker and Ginsburg (4) found that the antidiuretic substance in rat's serum was not present in plasma. Ames and van Dyke (5) concluded that the intraperitoneal technique employed by Birnie and associates (2) was an insensitive method and that the results were grossly at variance with those which they themselves obtained using an intravenous method of assay.

In the present work an attempt was made to discover whether human serum has any such antidiuretic action in man. The simplest way to accomplish this is to inject the serum, not into a rat, but intravenously into a hydrated human subject. One milliunit of Pitressin can usually be detected by both methods. The donor of the serum can be used for the assay, provided that a diuresis has been established if necessary after the serum has been withdrawn. As the results described by Lloyd and Lobotsky were

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obtained by the assay of serum not more than one hour after its withdrawal from the subject, that period of time was the longest allowed to elapse before re-injection of the serum in the present work.

METHOD

Normal human volunteers were studied. No food or fluid was taken for twelve hours before the experiment. Blood was withdrawn from an antecubital vein into sterilized centrifuge tubes and allowed to clot. It was centrifuged and the serum separated. As soon as the blood had been withdrawn, the subject drank a liter of water and, in most cases, received an intravenous injection of 250 ml. of 5 per cent dextrose solution in five minutes, so as to hasten the onset of the diuresis. Urine was passed every ten minutes and the volume and chloride concentration measured. In all cases it was found that by the end of an hour the urine flow was rising—the rate ranging from 4 to 10 ml. per minute—and the urine contained no glucose. The serum was then re-injected, and urine passed at ten-minute intervals for a further half-hour. Immediately after this injection the serum was assayed for antidiuretic activity in rats, by the method of Birnie *et al.*, with slight modifications. Wistar rats were used in groups of 3, three hydrating doses of water (5 ml./100 Gm. of body weight) being given by gavage at hourly intervals. Serum (1 ml./100 Gm. of body weight) was injected intraperitoneally into the first group, and saline[1] (1 ml./100 Gm. of body weight) intraperitoneally into the second, immediately after the third hydrating dose of water. (In practice, little time elapsed between the injection into the human subjects and into the rats: the former were always injected first.) The water load is the difference between total water given in the three doses and the urine volume excreted in the first two hours. The percentage of the water load excreted at ninety minutes was calculated; the difference between the percentage of the water load excreted by serum-injected and by saline-injected animals expresses the antidiuretic activity of the serum. In three experiments a third group of 3 rats was given 2 milliunits of Pitressin intraperitoneally.

All the human subjects used were tested for sensitivity to Pitressin and all showed, following the injection of 1 milliunit intravenously after previous hydration, a fall in the urine volume with a rise in chloride concentration in at least the next two ten-minute specimens.

RESULTS

Volumes of serum ranging from 3 ml. to 17 ml. were re-injected into the human subjects. In no case was antidiuresis observed. It is therefore pos-

[1] Physiological sodium chloride solution.

sible to give a maximum figure for the antidiuretic titer of each serum (Table 1), and in none of them was this as great as 0.1 milliunit of Pitressin per milliliter.

TABLE 1. THE ANTIDIURETIC ACTIVITY (ADA) OF HUMAN SERUM

Subject	ADA in terms of milliunits of Pitressin	Rat assay: ADA*	Rat assay: ADA of 2 milliunits of Pitressin*
1	<0.3	—	—
	<0.1	−19	—
2	<0.1	—	—
	<0.03	—	—
3	<0.1	−15	—
4	<0.1	−16	− 9
5	<0.1	−11	−19
6	<0.1	−20	−31

* Difference between percentages of water load excreted by test and control animals in 90 minutes.

Sera from 5 of these subjects were found to be moderately antidiuretic on injection into rats, showing that whatever substance produces this effect had not disappeared from the sera before they were re-injected into the human subjects. In 1 case this activity was greater than that produced by 2 milliunits of Pitressin and in 2 cases slightly less.

DISCUSSION

The antidiuretic activity found in these sera on rat assay was of the same order as that found by Lloyd and Lobotsky after dehydration. However, this assay clearly gives no measure of the antidiuretic activity in man, unless the rat is at least 20 times as sensitive to the human hormone as to Pitressin, a possibility which seems too remote to be entertained.

It has previously been reported that the serum taken from a man with hypopituitarism just before he showed an almost normal diuretic response to a water load, was found to be antidiuretic by rat assay, whereas that from a woman with Sheehan's disease in whom the diuretic response was absent showed no such activity (Chalmers and Lewis (6); Lewis (7)).

Brun, Knudsen and Raaschou (8) demonstrated antidiuretic activity in the blood of 3 men after fainting, by withdrawing from 200 to 450 ml. and transfusing it into hydrated normal subjects. They made no attempt

to assay by this method the amount of antidiuretic substance present, but inspection of one of their graphs (Fig. 3) shows that the transfusion of 200 ml. of blood reduced the urine flow of the recipient for forty minutes. This is the effect usually produced in a normal subject by the intravenous injection of 1 or 2 milliunits of Pitressin. The antidiuretic activity present in the blood after fainting, a condition which is known to be an extremely powerful stimulus for the release of antidiuretic hormone (Chalmers, Lewis and Pawan, 1953, in preparation), is therefore likely to be of the order of 0.01 milliunit per milliliter, in terms of Pitressin.

The probable explanation of these discrepancies is that the rat assay method is a nonspecific one, and that the antidiuretic effect is due to some substance present in the serum, such as that described by Dicker and Ginsburg (4), or to a stimulus such as pain.

SUMMARY

1. After twelve hours' dehydration, human serum was found to be antidiuretic when injected intraperitoneally into hydrated rats. The activity was of an order similar to that exerted by 2 milliunits of Pitressin per milliliter.

2. By re-injection into the donor, the activity was found to be less than that of 0.1 milliunit of Pitressin per milliliter.

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MINERAL COMPOSITION OF THERMAL SWEAT IN HEALTHY PERSONS

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RECENT investigations have shown that adrenocortical hormones significantly influence the mineral composition of thermal sweat in man; moreover, the chloride and sodium contents of sweat are claimed to be useful indices of adrenocortical function (1, 2, 3). Based on such observations, evidence has been given showing that acclimatization to heat is dependent on adrenocortical function (4, 5). The present writers have observed that heavy muscular work causes a significant decrease in the sodium and chloride contents of thermal sweat (6), possibly due to an increased adrenocortical function. This has been regarded as a purposeful reaction tending to conserve the salt reserves of the body during excessive sweating. It seemed to be of considerable interest to observe how far differences in physical fitness are mirrored in the mineral composition of thermal sweat, as might be expected on the basis of the results obtained in the studies on acclimatization to heat (4, 5). For this purpose, samples of thermal sweat were collected from persons representing different levels of physical activity; these samples were analysed for mineral composition.

MATERIAL AND METHODS

The material comprised 31 champion lumbermen, 19 soldiers, 17 men of sedentary occupations (such as physicians and students) and 69 nurses or nurse-students. The lumbermen had performed very hard work for several years and they were all first-class athletes. The soldiers were regarded as an intermediate group between the very fit lumbermen and the 19 totally untrained sedentary men.

Sweat samples were collected in plastic sleeves in the Finnish Saunabath and analysed for their chloride, potassium and sodium contents as described earlier (6, 7). This form of thermal stimulus aims at the application of the physiological maximum stimulation of sweating. The mineral composition of thermal sweat was compared in different groups, using

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samples collected in as uniform circumstances as possible. About 200 such samples were collected under normal and under various experimental conditions, and analysed to elucidate the mutual correlation of the contents of the different minerals in each sample.

RESULTS

Table 1 shows the mean values in the different groups. Corrected values for each mineral have been given for the group of lumbermen, because the majority of the samples from these men were collected during a four-day competition in the course of which the subjects were stressed to all-out performances. As heavy muscular work decreases the sodium and chloride contents of thermal sweat (6), all values obtained did not represent true resting values. In the subjects from whom the samples were obtained during the competition, the corrected values were calculated "backwards" with the aid of the average daily change (6).

Statistical analysis of the results revealed that some significant differences in the sodium or chloride values in the different groups were to be observed (Table 1). The mean values were, however, surprisingly similar, taking into consideration the large individual variations in each group. Furthermore, sex differences were noted in the concentration of sodium.

The potassium concentration did not differ significantly in the three male groups. On the other hand, the *t*-test revealed a statistically significant difference in the potassium values for the male and female subjects ($P < 0.001$). The ratios sodium:chloride, sodium:potassium and chloride:potassium showed statistically significant group and sex differences (Table 2).

Figures 1 and 2 illustrate the close relationship between the sodium and chloride contents of thermal sweat. The positive correlation was linear and highly significant for both men and women (men: $r = 0.977 \pm 0.0039$; and women: $r = 0.913 \pm 0.020$). The equations of the regression lines are somewhat different for male and female subjects, owing to a higher sodium:chloride ratio in the latter (men: $X = 56.28 + 1.05\ (Y - 45.05)$, $Y = 45.05 + 0.912\ (X - 56.28)$; and women: $X = 63.60 + 1.113\ (Y - 43.70)$, $Y = 43.70 + 0.746\ (X - 63.60)$). In spite of the fact that the sweat samples were collected from women under strictly identical conditions, the correlation is somewhat lower in them than in men. The very high correlation in men is especially noteworthy, because a considerable number of values represented in the graph were obtained under different experimental conditions, some of which have earlier been seen to affect significantly the mineral composition of thermal sweat (6, 7).

TABLE 1. MINERAL COMPOSITION OF THERMAL SWEAT IN DIFFERENT GROUPS

Group	No. of subjects	Mineral	Concentration (mEq./L.) M ± SD	Range	Significance of difference *vs.* other groups
Men	67	Na	58.42 ± 15.70	30.0–104.4	Women 0.05 > P > 0.01
Lumbermen	31		53.59 ± 14.26	30.0–104.4	Sed. men P > 0.05 Soldiers P > 0.05 Nurses 0.05 > P > 0.01
Men of sedentary occupation	17		61.06 ± 14.47	40.5– 94.0	Lumbermen P > 0.05 Soldiers P > 0.05 Nurses P > 0.05
Soldiers	19		60.71 ± 16.92	31.8– 93.5	Lumbermen P > 0.05 Sed. men P > 0.05 Nurses P > 0.05
Nurses	69		63.60 ± 19.33	21.3–129.0	Lumbermen 0.05 > P > 0.01 Sed. men P > 0.05 Soldiers P > 0.05
Men	67	Cl	45.39 ± 15.50	21.7– 84.0	Women P > 0.05
Lumbermen	31		39.78 ± 13.03	21.7– 81.0	Sed. men P > 0.05 Soldiers 0.05 > P > 0.01 Nurses P > 0.05
Men of sedentary occupation	17		46.22 ± 17.77	27.0– 84.0	Lumbermen P > 0.05 Soldiers P > 0.05 Nurses P > 0.05
Soldiers	19		50.40 ± 15.60	25.2– 78.2	Lumbermen 0.05 > P > 0.01 Sed. men P > 0.05 Nurses P > 0.05
Nurses	69		43.68 ± 15.78	12.3– 99.0	Lumbermen P > 0.05 Sed. men P > 0.05 Soldiers P > 0.05
Men	67	K	10.00 ± 2.39	5.9– 16.0	Women P < 0.001
Lumbermen	31		9.59 ± 2.46	5.9– 15.8	Sed. men P > 0.05 Soldiers P > 0.05 Nurses P < 0.001
Men of sedentary occupation	12		11.30 ± 2.84	6.8– 15.5	Lumbermen P > 0.05 Soldiers P > 0.05 Nurses P > 0.05
Soldiers	19		10.11 ± 2.13	7.1– 16.0	Lumbermen P > 0.05 Sed. men P > 0.05 Nurses 0.01 > P > 0.001
Nurses	69		12.22 ± 2.55	6.0– 18.1	Lumbermen P < 0.001 Soldiers 0.01 > P > 0.001 Sed. men P > 0.05

TABLE 2. RATIOS BETWEEN SODIUM, CHLORIDE AND POTASSIUM IN DIFFERENT GROUPS

Group	No. of subjects	Ratio	Mean ± S.D. (mEq./L.)	Significance of difference *vs.* other groups	
Men	90	Na:K	6.19 ± 2.67	Women	$0.05 > P > 0.01$
Lumbermen	31		5.86 ± 2.01	Sed. men	$P > 0.05$
				Soldiers	$P > 0.05$
				Nurses	$P > 0.05$
Men of sedentary occupation	12		6.04 ± 2.79	Lumbermen	$P > 0.05$
				Soldiers	$P > 0.05$
				Nurses	$P > 0.05$
Soldiers	19		6.32 ± 2.85	Lumbermen	$P > 0.05$
				Sed. men.	$P > 0.05$
				Nurses	$0.05 > P > 0.01$
Nurses	69		5.39 ± 1.95	Lumbermen	$P > 0.05$
				Sed. men.	$P > 0.05$
				Soldiers	$0.01 > P > 0.001$
Men	67	Na:Cl	1.32 ± 0.15	Women	$P < 0.001$
Lumbermen	31		1.38 ± 0.12	Sed. men	$P > 0.05$
				Soldiers	$0.01 > P > 0.001$
				Nurses	$0.05 > P > 0.01$
Men of sedentary occupation	17		1.36 ± 0.16	Lumbermen	$P > 0.05$
				Soldiers	$0.01 > P > 0.001$
				Nurses	$0.05 > P > 0.01$
Soldiers	19		1.22 ± 0.14	Lumbermen	$0.01 > P > 0.001$
				Sed. men	$0.01 > P > 0.001$
				Nurses	$P < 0.001$
Nurses	69		1.55 ± 0.29	Lumbermen	$0.05 > P > 0.01$
				Sed. men	$0.05 > P > 0.01$
				Soldiers	$P < 0.001$
Men	67	Cl:K	4.77 ± 2.26	Women	$0.01 > P > 0.001$
Lumbermen	31		4.44 ± 1.93	Sed. men	$P > 0.05$
				Soldiers	$P > 0.05$
				Nurses	$0.05 > P > 0.01$

TABLE 2—(*continued*)

Group	No. of subjects	Ratio	Mean ± S.D. (mEq./L.)	Significance of difference *vs.* other groups	
Men of sedentary occupation	12		4.49 ± 2.61	Lumbermen	$P > 0.05$
				Soldiers	$P > 0.05$
				Nurses	$P > 0.05$
Soldiers	19		5.42 ± 2.38	Lumbermen	$P > 0.05$
				Sed. men	$P > 0.05$
				Nurses	$P < 0.001$
Nurses	69		3.73 ± 1.52	Lumbermen	$0.05 > P > 0.01$
				Sed. men	$P > 0.05$
				Soldiers	$P < 0.001$

Figure 3 demonstrates the lack of correlation between the potassium and sodium contents. Neither was there any correlation between the potassium and chloride values.

DISCUSSION

A considerable number of observations indicate that the sodium and chloride contents of thermal sweat are definitely affected by the administration of desoxycorticosterone acetate or ACTH (1, 2, 3, 5) and by stresses known to increase adrenocortical function, such as heat (4, 5) and muscular work (6). In addition to the adrenocortical hormones, the composition of sweat is also affected by the rate of sweating (8, 9, 10) and by the skin temperature (11). In the present work, an attempt was made to apply a strong thermal stimulus in order to minimize the effect of varying rates of sweating, yet considerable differences between the individual sweating rates were observed. However, often the subjects showing a slow rate of sweating had the highest mineral concentrations in their sweat, a relationship opposite to that observed when varying rates of sweating are produced in a single subject (10). Thus, unless other unknown regulatory mechanisms independent of adrenocortical function influence the composition of the sweat, adrenocortical activity (mainly its "mineralocorticoid" function) can be conveniently assessed by the study of thermal sweat. The very high correlation between the sodium and chloride concentrations, as observed in the present work and also by Locke *et al.* (10), makes the determination of sodium concentration unnecessary, thus eliminating the need for an expensive flame photometer. The simple titrimetric chloride analysis gives the information required.

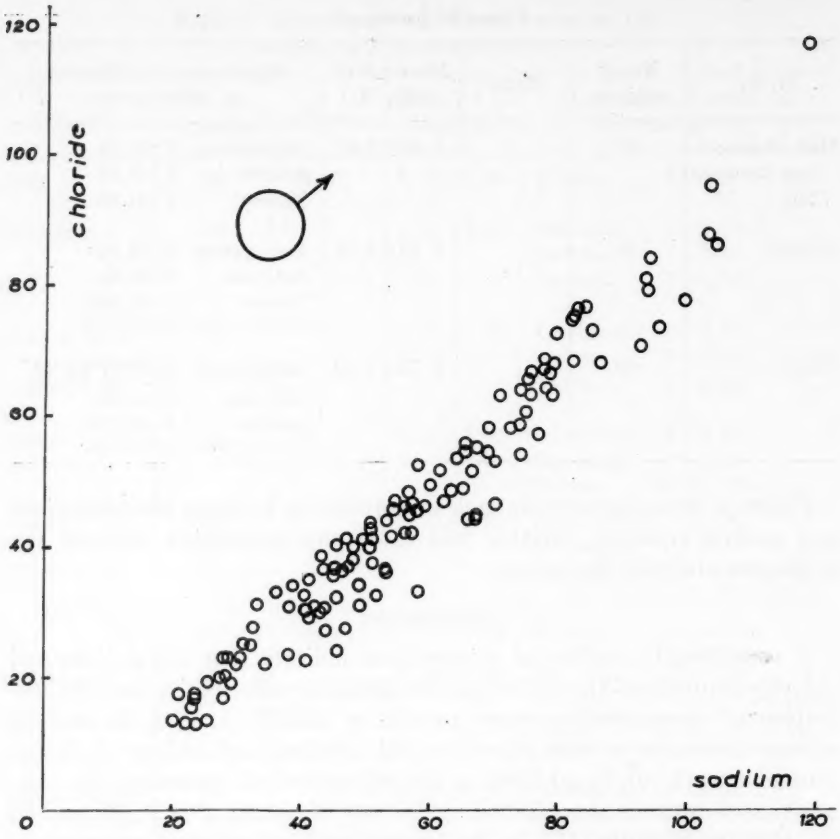

FIG. 1. Correlation between the chloride and sodium content of sweat in men. (All values in terms of mEq./L.)

If, under these conditions, the sodium and chloride concentrations are regarded as reliable guides to the physiological state of the adrenal cortex, the conclusion must be drawn that adrenocortical function at rest is of minor importance for the working capacity of man, and that many hard-working men have a much lower activity of the adrenal cortex than many men whose highest physical performance is restricted to driving daily some miles by car. In view of the facts which have accumulated in the animal experiments about the effect of prolonged stress on the adrenocortical function (12), this apparent paradox can be explained by assuming variable hormone secretion *at rest*, in spite of larger functional reserves in subjects accustomed to the continuous stress of hard physical work—a

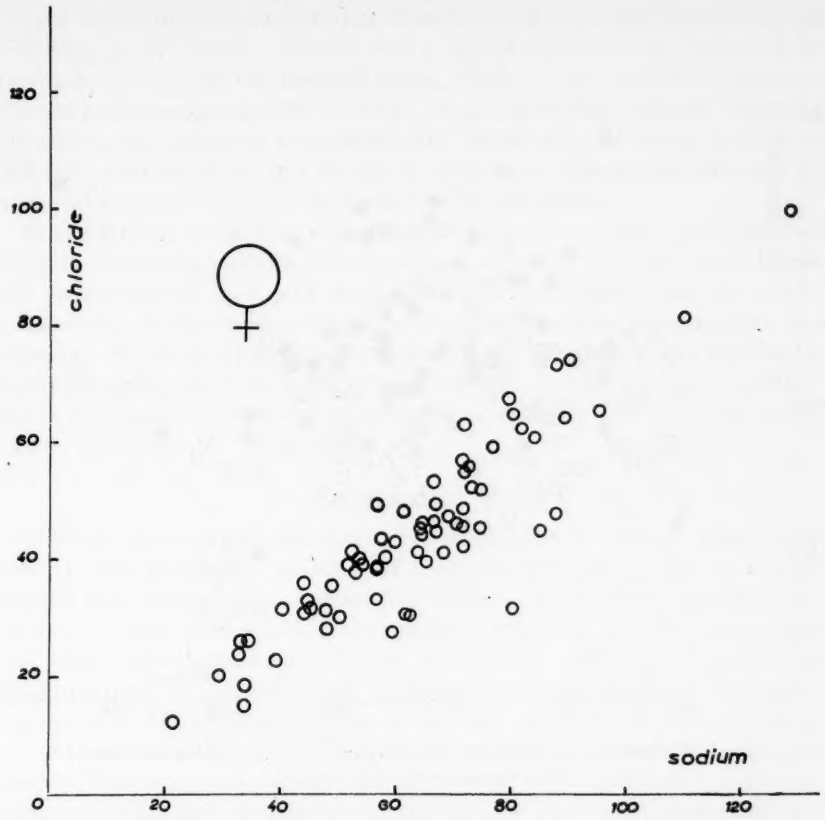

FIG. 2. Correlation between the chloride and sodium content of sweat in women. (All values in terms of mEq./L.)

theory also proposed by Locke *et al.* (7). Thus, although the functional capacity of the adrenal cortex may play an important role in the physical fitness of man, there seem to be hardly any differences in the resting activity of the adrenals of physically fit and unfit men.

Although a low sodium chloride value cannot be taken as an indicator of good physical fitness, it still seems possible that repeated sweat analyses in the same individual under training could give valuable information of his condition. Future research should proceed along such lines, to avoid the difficulty produced by the large individual variations. Such observations could also solve the hitherto unanswered question as to whether the chloride or sodium content of thermal sweat is a useful indicator of *acute* adrenocortical changes only, as is the number of circulating eosinophils,

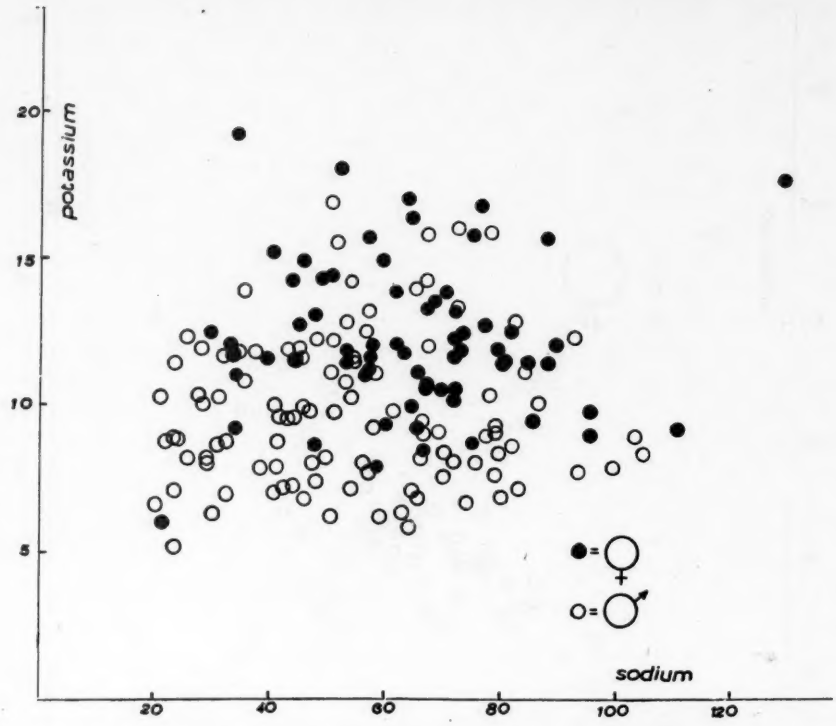

FIG. 3. Correlation between the potassium and sodium content of sweat. (All values in terms of mEq./L.)

or whether it can be used advantageously also in *long-term* studies. If the foregoing possibility should prove to be a reality, sweat analysis before and after a standard stress may give valuable information in long-term experiments.

The subjects in the different groups examined in the present study represent very different levels of physical activity, the lumberjack's caloric consumption being 5,000 to 6,000 calories per day (13) and that of a man in a sedentary occupation, about 2,000 calories per day. In spite of this fact, which is but one expression of the differences between the groups, the mean sodium and chloride values showed only small variations. Furthermore, sodium values up to 90 mEq./L. were observed in physically highly fit lumbermen. Therefore the conclusion seems to be justified that the sodium or chloride concentration of thermal sweat, unless distinctly outside the rather large physiological limits (from 10 to 100 mEq./L.), is no indicator of the physical condition of man.

It is interesting to observe that there exists a slight but significant sex difference in the sodium content, and a highly significant sex difference in the potassium content of thermal sweat. There is also a significant sex difference and some group differences in the ratios sodium:chloride, sodium:potassium and chloride:potassium. The significance of these findings is difficult to interpret. It may be due to a different chloride:lactate ratio in men and women (14) or to some other unknown factor.

In the present work, the potassium concentration showed no correlation with the sodium or chloride concentrations, whereas a negative correlation has been observed by Locke *et al.* (10). This discrepancy may be due to a difference in the material; the present observations were mainly obtained on different subjects, whereas in the work of Locke and associates, several experiments were made on each subject. It may also be noted that the potassium concentrations in the present work tend to be considerably higher than those of Locke *et al.*

SUMMARY

Chloride, sodium and potassium concentrations in thermal sweat have been studied in healthy persons, 67 men and 69 women. The men were divided into three groups representing different levels of physical activity. In spite of great differences in the physical fitness in the different groups only small differences were noted in the sodium, chloride and potassium concentrations of thermal sweat between the groups or sexes. This may indicate that the resting activity of the adrenal cortex is not greater in trained subjects doing daily hard physical work than in entirely untrained persons. The potassium concentration of sweat was significantly higher in women than in men. Furthermore, statistically significant group and sex differences were noted in the sodium:chloride, sodium:potassium and chloride:potassium ratios.

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FEMALE PSEUDOHERMAPHRODISM: A DESCRIPTION OF 2 UNUSUAL CASES

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SOMEWHERE in the no-man's land between endocrinology, morphology and genetics lie the hermaphroditic anomalies. Unfortunately, the relative influence of any single factor in a particular case is all too often in doubt; great confusion still exists as to the role of the "sex" hormones, of the organizers, and of the genes. Not only is the nature of the developmental abnormalities not understood but in many cases one is ignorant of the true sex of the child, since it cannot be assumed that the actual somatic sex is always the same as that of the gonads. Furthermore, it is often impossible to predict with any degree of confidence the the course of psychosexual development (1, 2).

Young (3) divided his patients into three groups, depending upon the status of the gonads. The true hermaphrodite was defined as the patient with both ovarian and testicular tissue. The male pseudohermaphrodite had testes and no evidence of ovaries but, depending upon the degree of the anomaly, might show hypospadias, cleft scrotum, cryptorchidism, a vagina leading into the urethra or into the perineum, and even a uterus and tubes. Contrariwise, the female pseudohermaphrodite had to have ovaries and no testes, but could have any other variation of the internal or external genitalia.

Departing from this strictly morphologic categorization, Wilkins (4) suggested a more functional classification into two groups: a) "hormonal sex reversal," due to the continued secretion of an excess of an inappropriate "sex" hormone by the endocrine system of the patient, the clinical picture depending upon whether the onset was fetal or postnatal, and b) "intersexes," due to abnormal genetic factors, to maternal hormonal or other influences acting upon the embryo, or to incomplete antagonism by the hormones of the fetal testis to the maternal estrogen and to the innate tendency toward feminization.

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Wilkins places in the "intersexes" those patients which Young termed "true hermaphrodites" and "male pseudohermaphrodites." He notes that all the female pseudohermaphrodites which have been described, with the possible exception of one, showed excessive adrenocortical production of androgen and hence were examples of "hormonal sex reversals." The clinical picture is fairly uniform; the ovaries are small and immature; the uterus, tubes, and vagina are present; however, the vagina does not open separately into the vulvar cleft, but joins the urethra instead to form a urogenital sinus which opens near the base of the clitoris. Infrequently the vagina does not communicate with the urethra, but remains a blind pouch. Wilkins described one child in whom the urogenital sinus penetrated the phallus.

We have recently encountered 2 instances of female pseudohermaphrodism which are interesting from several points of view. Both infants had urethras passing completely through the phallus—a rare condition, which has been reported previously in only 1 other patient (4)—leading to an erroneous diagnosis of male pseudohermaphrodism. One infant showed marked bilateral adrenal hypertrophy, but the other (5) had normal adrenals, no acne, no hirsutism, and normal urinary 17-ketosteroids (Holtorff-Koch). Accordingly, the latter patient is not an example of "hormonal sex reversal" in Wilkins' sense and is only the second reported case of female pseudohermaphrodism not associated with adrenal hyperplasia. Another interesting feature is that this same child was one of twins, the sibling being a normal female.

Patient #1, G.M., was born July 25, 1950, at another hospital, was discharged as a normal white male at five days of age, but was readmitted eight days later with vomiting, diarrhea, and dehydration. Despite intensive treatment, including adrenocortical therapy, the child failed to respond and was transferred to St. Christopher's Hospital for children at 39 days of age.

Admission physical examination revealed dehydration, subcutaneous wasting, a blood pressure of 80/60, a grade I harsh basal cardiac murmur, undescended testes, a normal penis, and a tubular-shaped midline abdominal mass. The BUN level was 25 mg. per 100 cc., the carbon dioxide combining power 42 volumes per cent, the serum chloride level 85 mEq./liter, and the hemoglobin 7 Gm./100 cc.

The child was given an evaporated milk formula with added salt, and blood transfusions. This was followed by clinical improvement, and the heart murmur disappeared. The serum chloride level returned to normal but the BUN remained elevated. Withdrawal of salt led to recurrence of dehydration and cessation of weight gain, although there was no vomiting or diarrhea at that time. The specific gravity of the urine ranged from 1.001 to 1.006. Intravenous urography showed poor visualization of the kidneys but good excretion into the bladder. Retrograde pyelography and cystography revealed no abnormalities of the genito-urinary tract. The 24-hour urinary excretion of formaldehydogenic corticoids was normal (0.2 mg.). Unfortunately the level of urinary 17-ketosteroids was not ascertained. In spite of continued supportive therapy the child again became dehydrated and weak, failed rapidly, and died at the age of 87 days.

At postmortem examination the body was first considered to be that of a fairly well developed, white, male infant. The anus was patent and normal. The penis was 2 cm. long and 1.5 cm. in diameter. The distal end was somewhat more bulbous than usual and bent slightly towards the ventral surface. There was moderate phimosis. The urethral meatus was seen at the tip of the glans. The scrotum was small and flaccid, and contained no testes.

In the abdomen no testes were present but, in relatively normal position, tubes, uterus and ovaries were found (Fig. 1, a and b). The uterus measured 3.2×0.6×0.6 cm. A small, short vagina, 1.8 cm. long, led from the cervix uteri to the urethra, entering at the site of the verum montanum. The right tube was 3.6 cm. long and the left, 3.5 cm. The right ovary measured 3.1×0.4×0.1 cm. and the left was 1.9×0.4×0.1 cm. The tubes and ovaries appeared normal, except for a thin-walled cyst containing clear fluid, in the left ovary. Microscopically the ovaries and tubes were normal, with small follicular cysts in both ovaries. At the site of juncture of the vagina and urethra, distinct prostatic tissue could be seen (Fig. 1,c). The left adrenal gland weighed 7.0 Gm. and measured 3.5×2.5×1.3 cm. The right adrenal weighed 9.5 Gm. and measured 5.0×3.3×1.6 cm. Both glands were coarsely nodular. Microscopically the nodules were noted to be adrenocortical adenomata, the majority of which seemed to consist of zona fasciculata tissue. All other endocrine glands were anatomically normal. Aside from patchy atelectasis in the lungs, the remainder of the postmortem examination revealed nothing abnormal.

The final anatomic diagnoses were: female pseudohermaphrodism, bilateral adrenal hyperplasia with multiple adenomata, and patchy atelectasis and interstitial pneumonitis of the lungs.

Patient #2, B.F., was born in April 1951, the second of premature colored twins. The first child was a perfectly normal female (4 pounds 8½ ounces in weight, and 18½ inches long); 4 other siblings were also normal. The second twin, the patient, delivered as a breech, weighed 4 pound 3 ounces, and was 18 inches long. Four membranes were distinguished in the placenta, and it was inferred that the twins were fraternal.

Immediately after birth, the patient appeared to be healthy and vigorous, except for an umbilical hernia and a perineal deformity (Fig. 2, a and b). The urethra passed through the large protruding phallus as indicated by the fact that urine was seen emanating from the distal end. The phallus lay within a horseshoe-shaped fold of tissue. About one centimeter behind the base of this organ were two perineal orifices. Feces were excreted through the posterior of these openings, and urine from the anterior one. Hence it was inferred that the most posterior orifice was the anus, and that the other two were urethral openings. Inasmuch as there was a functioning urethra penetrating the phallus, the patient was considered to be a male pseudohermaphrodite, with bifid scrotum, abdominal testes, and an anomaly of the urethra with perineal hypospadias.

Roentgenograms of the skull and long bones showed slight prematurity in bone age but were otherwise irrelevant. Repeated intravenous urography failed to demonstrate the kidneys or ureters, but did show a collection of dye in the bladder. Retrograde opaque cystography revealed filling of two viscera, one anterior to the other, which were thought to constitute a double bladder. Subsequently it was shown that the posterior viscus was a distended uterus, and the anterior, the bladder. The urinary 17-ketosteroid excretion was normal (0.11 mg. in twenty-four hours), and the BUN level was also normal (12 mg. per 100 cc.).

The child was discharged in August 1951 with the understanding that it was to be

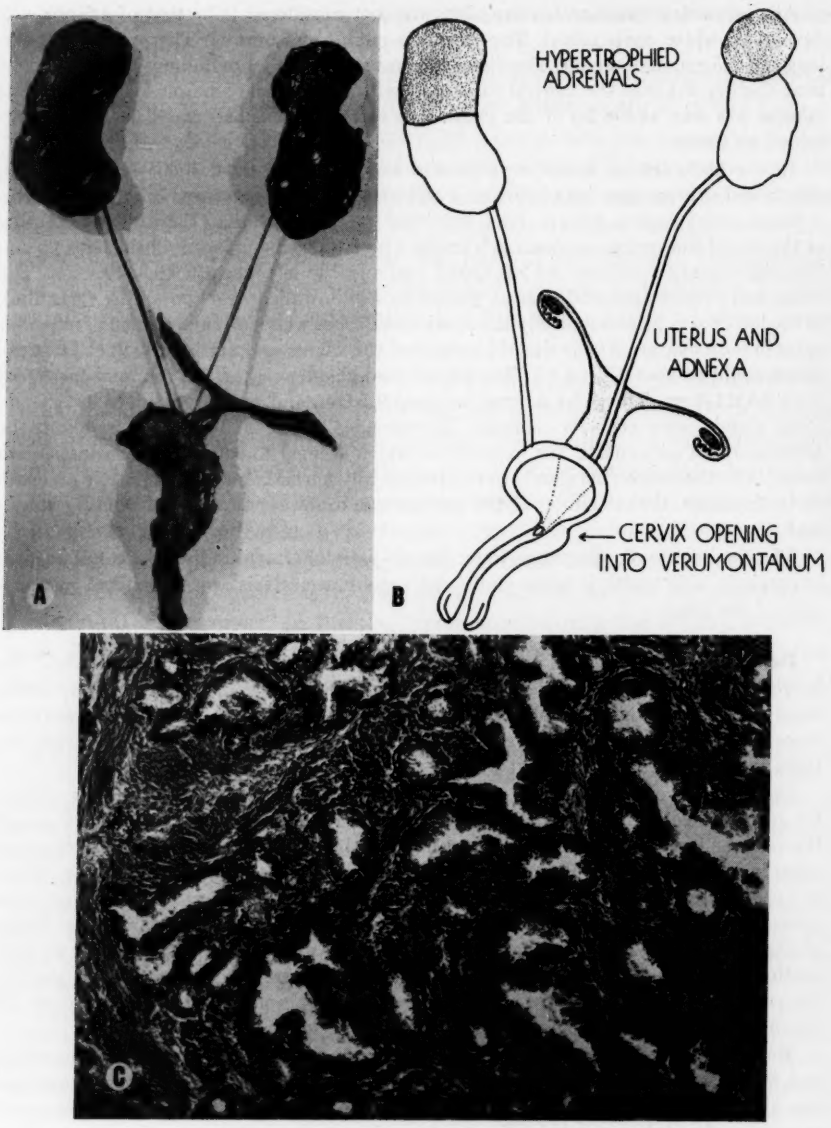

FIG. 1. *a*) Photograph of organs removed from Patient G.M.
b) Diagrammatic explanation of Fig. 1*a*.
c) Section of prostatic tissue, Patient G.M.

readmitted at a later date for confirmatory diagnostic surgical exploration and appropriate plastic surgery. Since there was no laboratory evidence of excessive adrenocortical function such as occurs in female pseudohermaphrodism, it was still believed that the child was a male.

The infant was readmitted to the hospital December 16, 1951, with a history of an upper respiratory tract infection, poor appetite, fever, and greenish stools. One generalized clonic convulsion had occurred that day. The baby was apathetic, crying only weakly. Rectal temperature was 103° F. and dullness and decreased breath sounds were

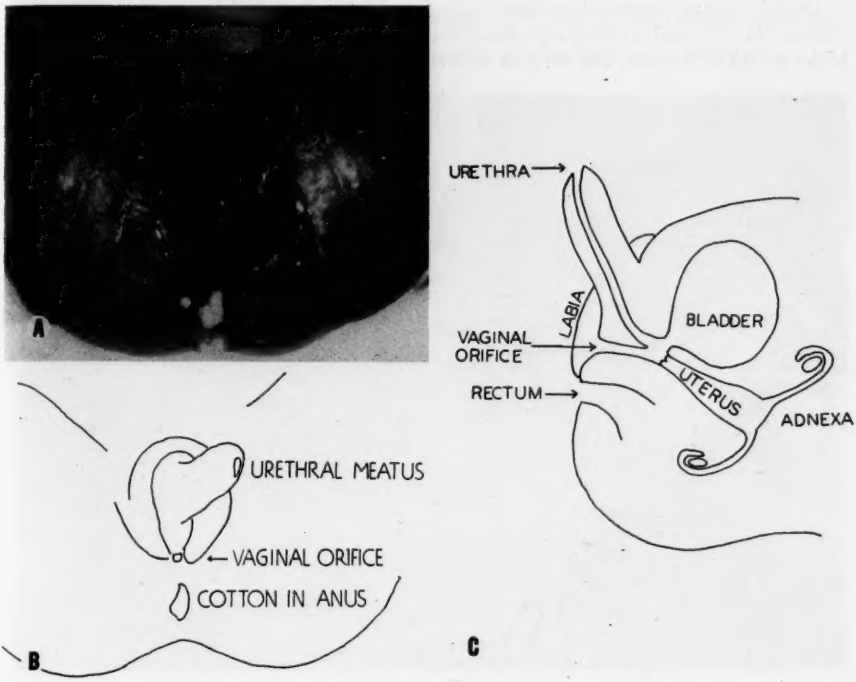

FIG. 2. *a*) Photograph of perineum of Patient B.F.
b) Diagrammatic explanation of Fig. 2*a*.
c) Diagram of autopsy findings, Patient B.F.

detected at the right lung base. Although the reflexes were generally hyperactive, there was no clonus, nor any signs of meningeal irritation. Spinal puncture was unrevealing. A blood culture was later reported to show pneumococcus. The patient died three hours after admission. The clinical diagnoses were pyelonephritis, pneumonia and male pseudohermaphrodism.

At autopsy, the previously described umbilical hernia and perineal anomaly were confirmed. The lungs were the seat of interstitial fibrosis and congestion. The kidneys were slightly enlarged and congested but otherwise normal. The pancreas was normal. Ureters were within normal limits. The bladder was larger than normal, with the wall

somewhat thickened, although the mucosa was normal. The urethra opened into a sinus running in front of the rectum (Fig. 2c).

The clitoris was 5 mm. in diameter and 1 cm. long. The uterus, which seemed dilated and thin-walled (2 mm. thick) was $1 \times 3 \times 4$ cm. in size. The tubes were 1-mm. cords of tissue leading from the uterus to the two ovaries, each of which measured $2 \times 2 \times 5$ mm. There was no discernible cervix; the uterus narrowed abruptly to a 2-mm. tract which joined the sinus (2-mm. diameter) into which the urethra also emptied. This sinus opened just in front of the rectum, and an extension from it passed through the clitoris, connecting the distal end of this organ with the bladder.

On microscopic examination many primary and Graafian follicles were noted in the ovaries (Fig. 3), and no testicular tissue could be discovered. The adrenals had a combined weight of 3 grams. The cortices were slightly thin, and microscopically there was a

FIG. 3. Section of ovary, patient B.F.

decrease in the normal vacuolization, consistent with some lipid depletion (Fig. 4). There were no adenomas. The thyroid and pituitary glands were normal. The brain was congested but otherwise normal.

The final anatomic diagnoses were: interstitial fibrosis of the lungs and pneumonia, congenital anomaly of the perineum with female pseudohermaphrodism and pneumococcus bacteremia.

DISCUSSION

There are many theories which attempt to explain the dynamics of hermaphrodism. The most popular of these is based upon the work of Wells (6) and others, which predicates that the basic configuration is fe-

male, and that male structures are formed under the influence of masculinizing hormones secreted by the embryonic testes. Accordingly, a genetic male would be expected to retain the basic feminine characteristics, and maleness would not develop if the embryonic testicular hormone were deficient or rendered ineffective by excessive maternal female hormone. Accordingly, an excessive amount of androgen produced by the embryonic adrenals, or possibly by the mother, would be an explanation of the female pseudohermaphrodite.

We cannot reconcile Patient #2 with this theory, as there were no recognizable signs of excessive androgen production—no hirsutism, no

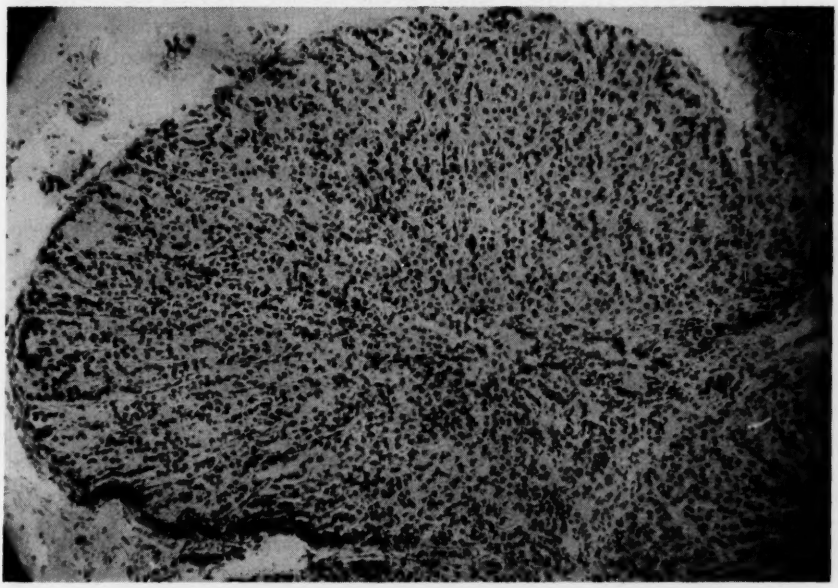

FIG. 4. Section of adrenal gland, Patient B.F.

acne, no advance in bone age, no adrenal hypertrophy, and no increase in 17-ketosteroid excretion. It might be postulated that a temporary androgenic excess occurred at a critical period in the development of the fetus, but this must be regarded as a supposition shaped to fit the theory. The presence of a normal female twin reduces the possibility of maternal influences. Furthermore, we have recently seen a male pseudohermaphrodite with hypospadias and bilateral cryptorchidism, proven at autopsy, who had a normal twin. It therefore seems to us that genetic or organizer abnormalities, or at least nonendocrine influences, must have been operative in this case.

Since female pseudohermaphrodism may occur in the absence of androgenic excess, it follows that this hormonal abnormality is not required for the development of the anomaly described here. We have no alternative theory to offer, but we suggest that current thoughts on the etiology of pseudohermaphrodism should be amended.

SUMMARY

Two unusual instances of female pseudohermaphrodism are described in which the urogenital sinus penetrated the clitoris. These constitute only the second and third reported cases. One of the infants had bilateral adrenal hyperplasia. The other infant is the second reported case with no evidence of adrenocortical hyperplasia. The dynamics of pseudohermaphrodism are discussed briefly, and it is suggested that current theories do not explain the facts adequately.

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ADRENAL CORTICAL INSUFFICIENCY ASSOCIATED WITH EOSINOPENIA: REPORT OF A CASE

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INTRODUCTION

BECAUSE the level of circulating eosinophils can be quickly and inexpensively determined, such testing has been helpful in the diagnosis of adrenal cortical insufficiency. There has, however, been some controversy concerning the normal range of circulating eosinophils and concerning the interpretation of eosinophil response tests. A case of Addison's disease is reported in which the eosinophil count was 25 per cu. mm. before therapy. This report illustrates the fact that, although the eosinophil count is almost always normal or elevated in the presence of adrenal cortical insufficiency, eosinopenia does not exclude the possibility of Addison's disease.

CASE REPORT

History: This 38-year-old colored man was admitted to St. Anthony Hospital on August 15, 1952. He had been listless for four months and had lost 40 to 50 pounds during this period. Other complaints were anorexia, extreme weakness and loss of sexual libido (all of three weeks' duration); vomiting of two days' duration; and intermittent right temporal headache of two or three weeks' duration.

Examination: Examination on August 16, 1952 revealed a somewhat somnolent patient who showed evidence of much recent weight loss and who appeared to be acutely and chronically ill. The temperature was 102° F. (orally), the blood pressure was 80/60, and the pulse was regular at a rate of 90. There was a diffuse blue-black discoloration of the oral mucous membranes, and the palmar creases were deeply pigmented. The heart and lungs were normal. The abdomen was normal, except for an inguinal hernia which was easily reducible. The neurologic examination was not remarkable, except for somnolence as previously mentioned.

A roentgenogram of the chest revealed fine diffuse mottling throughout both lung fields. The heart shadow was small.

Clinical course and laboratory findings: A tentative diagnosis of Addison's disease was made and 25 mg. of ACTH was given with the intention of performing a Thorn test. About the time of the ACTH injection the patient began to have difficulty in speaking and a few minutes later was disoriented. The eosinophil response test was therefore interrupted and the patient was given 100 mg. of cortisone intramuscularly, 10 mg. of desoxycorticosterone acetate (DCA) intramuscularly, and adrenal cortical extract (5 cc. intramuscularly and 5 cc. intravenously). Normal salt solution with 5 per cent glucose was started intravenously and a volume of 3,000 cc. was given in the next eight hours.

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A blood specimen drawn prior to therapy revealed 25 eosinophils per cu. mm.[1] Ten hours after initiation of treatment the patient was mentally alert and his temperature had dropped to 99.5° F. The blood pressure had risen to 90/70 and he stated that he felt "much stronger." There was no further vomiting. He was started on 12 to 15 grams of salt daily and hormonal therapy was withdrawn in order to facilitate confirmation of the diagnosis. Forty hours after the 100-mg. dose of cortisone the eosinophil count was 0. The patient received no treatment on August 17 or 18, except liberal amounts of oral fluids and the extra salt just mentioned.

All fluids were withdrawn at 6:00 P.M. on August 18. He was given 1,300 cc. of water between 8:00 A.M. and 8:10 A.M. (August 19). The overnight urine specimen (10:00 P.M. to 8:00 A.M.) measured 262 cc. The 8:00–9:00, 9:00–10:00 and 10:00–11:00 A.M. specimens measured 25, 75 and 80 cc., respectively. The withdrawal of fluids was followed by a rise in temperature to 102° F. and an increase in lethargy and weakness. The blood specimen drawn for sodium and potassium determination before therapy (August 16) was not satisfactory. On August 18 the serum sodium concentration was 131 millequivalents per liter and the potassium was 6.25 millequivalents per liter (a ratio of 21 to 1).

The reaction to a tuberculin patch test was negative on August 17 (after forty-eight hours). The result of a 1:10,000 intradermal tuberculin test was negative after forty-eight hours on August 20. Sputum showed no acid-fast bacilli on August 17, but the sample consisted largely or completely of saliva and was not considered satisfactory. Gastric washings showed no acid-fast bacilli on three occasions (cultures of this material gave negative results at a later date). No acid-fast bacilli were found in the spinal fluid. This fluid contained 31 lymphocytes and 6 polymorphonuclear leukocytes per cubic millimeter; the protein concentration was 53 mg. per 100 cc. and the glucose, 21 mg. per 100 cc. Four blood cultures showed no growths.

Following the completion of the water test, hormonal therapy was reinstituted. During the period August 19 to August 23 the patient received 10 mg. of DCA daily and from 25 to 50 mg. of cortisone daily except for August 21, when he received 75 mg. of cortisone. During this period he was lethargic and the temperature ranged between 101° and 102° F.

Streptomycin, 1 gram twice daily, was started on August 22, because of the likelihood of tuberculosis, and because of the intention of increasing the dose of cortisone. On August 23 the cortisone dose was increased to 100 mg. daily (2×50 mg., orally). On August 24 the serum sodium concentration was 123 millequivalents per liter and the potassium, 5.8 milliequivalents per liter. During the period August 23 to August 29 he received 75 to 100 mg. of cortisone daily and 10 mg. of DCA daily. He became afebrile on August 24 and remained so for the rest of the hospital course, except on occasions when attempts were made to reduce the daily dose of cortisone below 62.5 mg. On August 27, para-amino-salicylic acid was started in a dosage of 12 grams daily.

On September 12 a roentgenogram was interpreted as showing bilateral calcification of the adrenals.

After August 23 the patient improved steadily. During the following two months he gained 25 pounds in weight. On October 29 a chest film showed "almost complete" clearing of the lung infiltration and a definite increase in heart size.

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[1] The count was made on venous blood by the method of Randolph (1). It was repeated and confirmed by another experienced observer.

DISCUSSION

Although the history and physical examination suggested adrenal cortical insufficiency, the eosinopenia came as a surprise and cast some doubt on the diagnosis until more laboratory confirmation was available. Factors favoring the diagnosis of Addison's disease were: the characteristic history, the low blood pressure, the pigmentation, the markedly positive result of the water test, the marked abnormality of the serum sodium-potassium ratio, and the x-ray findings of small heart size and adrenal calcification. The response to therapy confirmed the diagnosis, as did the clinical relapse at the time of fluid restriction (water test). Since the mental confusion quickly responded to hormonal therapy we concluded that the central nervous system symptoms were due to Addison's disease and not to tuberculosis. Although the tuberculosis organism was never identified, a diagnosis of tuberculosis was justified for several reasons, including the x-ray findings (lungs and adrenals) and the fact that the lung findings disappeared after treatment with streptomycin and para-amino-salicylic acid. It is possible that the response to the intradermal skin test was modified by cortisone. The high cortisone requirement was in keeping with the general experience that higher doses of hormone are necessary in Addisonians with active tuberculosis.

COMMENT

In a group of 33 cases reported by Thorn (2) the lowest eosinophil count was 94. He concluded that, in the absence of complicating factors such as hypopituitarism or hypothyroidism, "a low level of eosinophils, *i.e.*, 50 to 80 cells per cu. mm., is strong evidence against the presence of adrenal cortical insufficiency."

Although eosinophil counts and eosinophil tests have proved to be useful laboratory aids, caution should be exercised in their interpretation. Some of the many factors which make interpretation difficult are the wide range of normal values (3), the diurnal variations (4), the fluctuations in relationship to feeding and fasting, and the variations in normal patients from day to day and hour to hour under nonstressful situations (5).

Bonner has demonstrated a diurnal variation in the eosinophil level of Addisonian patients (6).

A case of Waterhouse-Friderichsen syndrome was recently reported (7) in which the eosinophil count was 25. It is possible that the eosinopenia was initiated by the stress which preceded the adrenal changes and that insufficient time had elapsed to allow the re-establishment of a normal or elevated level (the count was made only a few hours after the adrenal changes were presumed to have occurred). It is nevertheless important

to realize that eosinopenia is possible in the presence of acute or chronic adrenal cortical insufficiency.

SUMMARY

A case of Addison's disease is presented with an initial eosinophil count of 25 per cu. mm.

Some of the extra-adrenal factors which influence the eosinophil level are mentioned and caution is urged in the interpretation of the eosinophil count.

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HYPOTHYROIDISM DUE TO THYROTROPIN DEFICIENCY WITHOUT OTHER MANIFESTATIONS OF HYPOPITUITARISM

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HYPOTHYROIDISM is generally considered to be produced by a lack of an adequate blood level of thyroid hormone, which results either from a primary defect within the gland itself or from the failure of the anterior pituitary to stimulate normal function of the thyroid. The latter condition, known as pituitary myxedema or secondary hypothyroidism, usually follows loss of function of the adenohypophysis due to tumor, necrosis or other destructive processes (1, 2, 3). In addition to the findings of myxedema, there are those associated with adrenocortical and gonadal insufficiencies due to the loss of tropic hormones from the pituitary (2). The present case of hypothyroidism does not fall into the foregoing classification; the patient was a severe diabetic in whom the adrenocortical responses and follicle-stimulating hormone levels were normal. The administration of thyroid-stimulating hormone demonstrated the presence of a physiologically intact thyroid gland, despite findings of hypothyroidism. The data indicate the unique occurrence of a selective depression or absence of the thyrotropin-producing cells of the adenohypophysis of this patient.

Case History

Mrs. L. H., a 64-year-old colored female, was studied at Temple University Hospital first in 1946. At that time, she was found to have diabetes mellitus of seven years' duration, diabetic neuropathy and depressive psychosis. A clinical diagnosis of myxedema was suggested but the B.M.R. was found to be −8 per cent. In view of the neuropsychiatric disorder, this finding was not reliable. Other data obtained at this time revealed a serum cholesterol value of 410 mg. per 100 cc., a negative serologic reaction, and a normal blood count and urine; the urea clearance was normal.

Diabetes mellitus was brought under control with diet and 38 units of protamine zinc insulin daily. Following discharge, she attended the clinic irregularly and gained weight progressively despite poor diabetic control. She failed to attend the psychiatric clinic for the proposed treatment program. However, three years prior to her second admission in 1952 she was treated at another hospital with electroshock therapy, during which a fracture of the second lumbar vertebral body was sustained.

Second admission. On January 3, 1952, the patient returned to the clinic after an ab-

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sence of six months, complaining of marked polyuria and thirst. She was found to be an obese, lethargic, withdrawn woman exhibiting sluggish responses and slow speech. Examination of the retina disclosed a mixed diabetic and hypertensive retinopathy with many scattered punctate hemorrhages, absorbing exudates and generalized attenuation and sclerosis of the arterioles. The blood pressure was 190/110; the heart was enlarged to the left. No thyroid tissue could be palpated in the neck.

The results of initial laboratory studies were normal except for the finding of glycosuria, acetonuria and hyperglycemia. These studies included a blood count, and determination of the B.U.N., total protein and A/G ratio, blood cholesterol and CO_2 combining power. The electrocardiogram revealed ST segment and T wave changes suggestive of left ventricular hypertrophy; a roentgenogram of the chest disclosed left ventricular enlargement.

During this initial period, diabetic stabilization was achieved with large doses of insulin. She received a mixture of NPH (140 units) and regular insulin (10 units) in the morning, NPH (80 units) at supper, and a diet consisting of P 70, F 60 and C 150 grams. On this program, blood sugar values were: fasting, 130 mg.; 11:00 A.M., 275 mg.; 3:00 P.M., 255 mg.; and fasting next morning, 128 mg. per 100 cc.

Because of her persistent depressive state, a course of electroshock therapy was instituted by the neuropsychiatric department. Following this, the patient became cheerful and, at times, garrulous for a period of one week. Gradually, she lapsed into her former depressed state. It is of interest that her insulin requirements decreased to 120 units, from the 230 units which she received prior to shock therapy.

At this time, the diagnosis of myxedema was suggested because of the patient's appearance and her sluggish responses. She refused to cooperate for a B.M.R. determination but submitted to a radioactive iodine tracer study performed by Dr. R. Robbins. Following administration of an 80-microcurie dose of I^{131}, the thyroid gland in twenty-four hours accumulated only negligible quantities of iodine. This study was repeated eight days later with similar results.

At this point, the patient was given 30 mg. of desiccated thyroid daily and discharged to the clinic receiving 100 units of NPH and 20 units of regular insulin, mixed.

Third admission. At home, the patient had refused to take any medications and was readmitted to the hospital on March 6, 1952, because of diabetic acidosis. Laboratory findings demonstrated glycosuria and acetonuria; the blood sugar level was 327 mg. and the cholesterol 305 mg. per 100 cc.; the CO_2 combining power was 38 volumes per cent. Diabetic control was rapidly re-established with regular insulin, following which control was stabilized on 140 units of NPH insulin daily. On this program the urine was virtually devoid of sugar. An insulin tolerance test, using 8 units of regular insulin, revealed the following values: fasting blood sugar (140 units of NPH on preceding day) was 100 mg.; 20 minutes, 90 mg.; 40 minutes, 104 mg.; 60 minutes, 90 mg.; 90 minutes, 94 mg.; and 120 minutes, 93 mg. per 100 cc. X-ray examination of the skull showed a normal pituitary fossa. The visual fields, exophthalmometric and B.M.R. readings were unsatisfactory because of inability of the patient to cooperate.

The status of the adrenal cortex was evaluated by means of the eosinophil responses to epinephrine and ACTH. The urinary 17-ketosteroid excretion (4, 5) was measured on three occasions; the urinary excretion of corticosteroids (6, 7) was also measured. These values are shown in Table 1, together with the follicle-stimulating hormone assay.

A third tracer dose of I^{131} was given, measurements being made of the accumulation gradient, the thyroidal iodine clearance, and the 24-hour thyroidal iodine uptake. The abnormally low results, together with the low absolute levels of radiothyroxine found

TABLE 1. PITUITARY AND ADRENOCORTICAL RESPONSES

		Eosinophils per cu. mm.	
		Fasting	Four hrs.
Epinephrine (1:1000)	0.3 mg.	111	22
ACTH	25 mg.	200	33

17-Ketosteroids in 24-hour urine:
Feb. 6, 1952 —4.1 mg.
Feb. 10, 1952 —8.4 mg.
March 15, 1952—7.1 mg.

Corticosteroids in 24-hour urine—1.2 mg. (as compound E)
Gonadotropic hormone excretion—212 mouse units.

in the blood, were consistent with the diagnosis of myxedema. One week later, she was given a course of thyroid-stimulating hormone (Armour's), using 30 mg. daily for three consecutive days in an effort to evaluate further the cause of the myxedema (1). A striking rise in radioactive iodine uptake following an 80-microcurie dose was found at the conclusion of the thyrotropin administration. The results of the tracer studies are shown in Table 2.

On the basis of these findings it was concluded that the patient could not be suffering from primary myxedema. The normal 17-ketosteroid values and normal eosinophil responses to epinephrine and ACTH indicated that the adrenal cortex and the portion of the anterior pituitary concerned with ACTH elaboration were not affected. Because of the latter findings, it was felt that desiccated thyroid therapy could be safely reinstituted. The initial dose was 30 mg., given three times daily. This dose was increased gradually to 60 mg., given four times daily. Careful observations were made of the pulse rate and blood pressure during this period. The pulse rate remained between 72 and 86 per minute and the blood pressure was stabilized at 150/80, which was somewhat lower than pre-

TABLE 2. RESULTS OF RADIOACTIVE IODINE TRACER STUDIES

	24-Hour thyroidal uptake %	1-Hour thyroidal uptake %	Thyroidal iodine clearance, cc./min.	24-Hour conversion ratio (PBI/I) %
Before TSH				
Feb. 4, 1952	1.0	—	—	—
Feb. 12*	6.1	6.0	5.0	—
Feb. 17*	7.0	6.6	12.5	4.7
After TSH, 30.0 mg. daily for 3 days				
Feb. 27, 1952	68.0	27.0	124.0	48.0

* The absence of any change in uptake from 1 hour to 24 hours suggests that these values represent tissue background count.

vious levels. During the period of thyroid therapy, no change was noted in the mental state of the patient. The insulin dose was gradually decreased to 40 units of NPH daily during the period of increasing thyroid dosage. The urine remained virtually free of sugar, based on fractional urine sugar determinations; however, the fasting blood sugar level became somewhat elevated to 194 mg. per 100 cc. At this point, the patient was transferred to a psychiatric hospital, with recommendations to continue the desiccated thyroid therapy at the established dosage level and to continue treatment with insulin and diet as outlined.

DISCUSSION

The clinical diagnosis of myxedema, when first suggested for this patient, was questioned because of the severity of her diabetes. However, the repeated iodine tracer studies left little doubt as to the accuracy of the bedside impression. Because of the unusual nature of these findings, it was decided to conduct a thorough investigation of the possible associated endocrine abnormalities.

The evaluation of the adrenal cortex by means of 24-hour urinary 17-ketosteroid and corticosteroid determinations, by the eosinophil response to epinephrine and ACTH, and by the insulin tolerance test revealed that no impairment of the functional integrity of this organ was present. Also, the severity of diabetes mellitus in this patient militated against adrenal cortical insufficiency, although these two conditions may coexist. The studies of adrenal cortex function were conducted because of the frequency with which insufficiency of this gland is encountered in myxedema. The observations also served to exclude the possibility of hyperadrenocorticism, which has been shown to produce a reduction of thyroid function, as indicated by a lowered rate of thyroidal accumulation of I^{131} and a lowered level of organic iodine in the blood (8, 9, 10).

Hypopituitarism in diabetic patients has been reported in 8 instances in the literature (11, 12, 13). In each of these, the diabetes was markedly ameliorated and, with 1 exception, insulin was discontinued. The studies of pituitary function in the present patient revealed no evidence of insufficiency with respect to adrenocorticotropin or follicle-stimulating hormone, nor was hypoglycemia produced by the intravenous insulin tolerance test. These data served to exclude the diagnosis of pituitary myxedema which, conceivably, may have complicated the diabetes.

Although there was evidence of cardiac disease, no signs of congestive failure or of fluid retention were observed. These factors should be excluded before attempting to interpret thyroidal iodine-clearance studies (10), since an increase in the extracellular fluid would expand the available space for iodine distribution and lead to low clearance rates. At the time of the performance of the tracer studies glycosuria was absent, so that urinary losses of iodine due to glycosuric diuresis could not have occurred. There was no evidence of renal functional impairment.

The administration of thyroid-stimulating hormone, after observing a negligible uptake of I^{131} on three occasions, resulted in remarkable changes in the final tracer study. These latter values fell within the hyperthyroid range, demonstrating the presence of physiologically intact thyroid tissue. This result suggested an isolated defect in the formation of thyrotropin within the anterior pituitary, since the other functions of the pituitary were not disturbed.

Purves and Griesbach (14) have shown by means of cytochemical studies that thyrotropin production in the rat pituitary occurs in those cells containing glycoprotein granules. They concluded that thyrotropin, adrenocorticotropin and gonadotropic hormones arise from several distinct cell types having different spatial distribution within the anterior pituitary. The existence of cells devoted solely to the elaboration of specific hormones in the gland permits the suggestion that one group of the basophilic cells may be adversely affected by metabolic, vascular or neurohumoral alterations which would produce an isolated defect of the type proposed in this case. Examples of a similar defect may be found in patients with hypogonadotropism or in those with idiopathic adrenal cortex atrophy responding to ACTH. In this connection, it may be of interest to study the effect of uncontrolled diabetes upon the ability of the pituitary basophils to produce glycoprotein granules in experimental animals, or upon the thyroidal iodine clearance and uptake in the human diabetic.

SUMMARY

The unusual findings of severe diabetes and myxedema occurring simultaneously in a patient led to an inquiry into the mechanisms underlying the disturbed thyroid function. Myxedema was first diagnosed on clinical grounds and the diagnosis was confirmed by the finding of a negligible thyroidal I^{131} uptake, and a low thyroidal iodine clearance and conversion ratio on three occasions after diabetic control was established.

Normal adrenocortical activity was indicated by the results of steroidal hormone excretion studies and eosinophil responses to epinephrine and ACTH.

Pituitary function with respect to follicle-stimulating hormone and ACTH production was found to be normal.

The administration of thyroid-stimulating hormone resulted in a marked increase in I^{131} uptake by the thyroid gland, and a high thyroidal iodine clearance and conversion ratio.

It was concluded that the myxedema manifested in this patient must have been the result of an isolated thyrotropin deficiency, since other functions of the pituitary were intact and the thyroid gland responded normally to exogenous thyrotropic hormone.

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HEMANGIOENDOTHELIOMA OF THE THYROID

REVIEW OF THE LITERATURE AND REPORT OF A CASE

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ONLY 2 cases of hemangioendothelioma of the thyroid have been described in American patients (1, 2). In Switzerland, on the other hand, this form of malignant goiter occurs in 16.2 per cent of thyroid cancers (Matti (3)). We recently had the opportunity to study such a tumor in the Hertzler Clinic.

CASE REPORT

A 67-year-old white female was admitted to the Halstead Hospital on July 7, 1951. She stated that in 1931 she had a goiter operation in another hospital and got along very well until February 1950, when the right side of her neck began to enlarge steadily and became harder. She did not notice rapid heart beat or shortness of breath. Her appetite was good but she had lost 30 pounds of weight during the past year.

Family history: The father died at the age of 79, the mother at the age of 30, of unknown cause. One brother died of cancer. The patient had five full-term pregnancies; the children are living and well. One of the daughters has a goiter.

Physical examination revealed a well developed and well nourished white female of the stated age. A large mass was found on the right side of the neck. It was movable and felt cystic and nodular to touch; there was no evidence of fixation to the surrounding tissues. The left lobe of the thyroid was slightly enlarged and felt nodular. No tumors were noticed in the breast. The lungs were clear to auscultation and percussion. The clinical impression was recurrent nodular goiter.

At operation (by VEC) on July 23, 1951 under local anesthesia, a portion of the previous scar was removed. A definitely encapsulated tumor, about the size of a hen's egg, was found on the right side of the thyroid. At the lower pole of the right lobe were several colloid nodules. The encapsulated adenoma and the colloid nodules were removed, leaving a small posterior portion of the right lobe. A nodular colloid enlargement was exposed on the left side and most of the left lobe was removed.

Pathologic report: The tumor removed from the right side of the neck weighed 65 grams and the two pieces of the left lobe weighed 20 grams. The solitary tumor had an oval form and measured 6 cm. in length and 4.5 cm. in diameter. The surface of the tumor was smooth and of yellowish brown color. Fragments of thyroid tissue were attached to the capsule in two places. Cross section revealed a large cystic cavity filled with light-brown coagulated fluid and some fresh red blood. The cavity was surrounded with a zone of rubber-like consistency, which contained numerous irregular slit-like spaces filled with dark red fluid. The cyst wall was of yellowish gray color, very firm

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in consistency, and homogeneous. Its thickness varied between 4 mm. and 8 mm. No penetration of the capsule by tumor tissue could be seen.

The two pieces of the left lobe measured 5×1.5 cm. and 4×2 cm., respectively. They consisted of small nodes between 6 and 11 mm. in diameter. Their cut surface was moist, translucent, and of light brown color.

Microscopic examination revealed a well encapsulated tumor. The thin fibrous capsule was not invaded by tumor cells, and no invasion of blood vessels was seen. The cystic cavity contained a network of coagulated plasma which stained pink with eosin, yellow

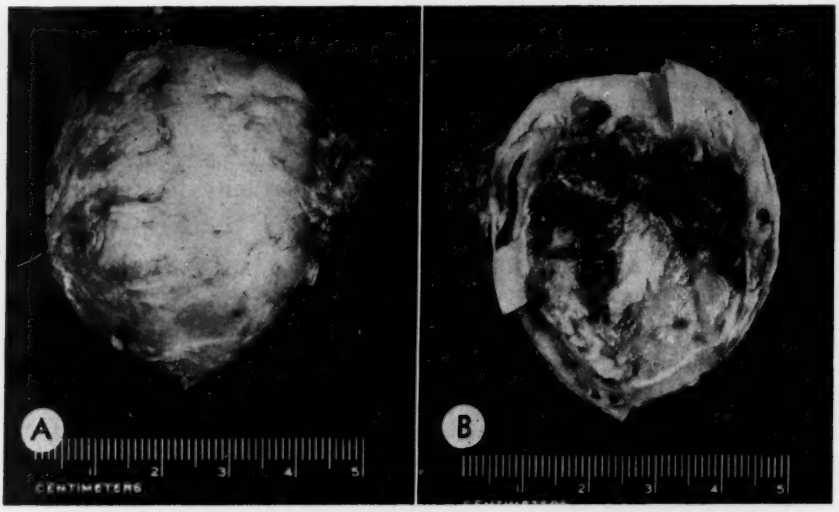

FIG. 1. Surgical specimen, 65 grams. A. The surface of the cystic tumor is smooth. B. The cut surface shows a hemorrhagic cavity surrounded with "rubber hyaline" and a tumor wall between 4 and 8 mm. thick.

with Van Gieson's stain, and blue and red with Mallory's anilin blue stain. The cavity was surrounded with a homogeneous, hyaline, pale-staining zone in which fibrocytes and groups of cavernous blood-filled lacunae were present. The zone was continuous toward the periphery, with tumor tissue consisting of many small oval or round spaces lined with atypical cells. The nuclei of these cells were of round, oval, or spindle shape, and had one or two nucleoli. The cytoplasm stained pale red. There was marked variation in the size and shape of the nuclei; occasional giant sized nuclei were noticed. No mitotic figures were found. Some of the spaces contained red blood cells, others were invaded by tumor cells with long cytoplasmic processes joining other cells or advancing to the opposite wall of the lumen. In this way, lattice-like structures were produced as described by Hedinger (4). The cell borders of the tumor cells were indistinct, and syncytial cell masses were occasionally encountered. Many of the cells had a vacuolated cytoplasm or contained large clear cavities, the nuclei being pushed to the cell border. The vacuoles did not stain with Sudan in frozen sections.

A small group of thyroid acini was recognized in a single area of the tumor. In several sections deposits of light brown pigment were noticed, either in macrophages, or free in the fibrous stroma. Near the capsule the tumor had the appearance of a compact spindle

cell sarcoma. The cells varied in size, the nuclei were of oval form, and the cells and collagenous fibers were arranged in fascicles and strands. Also, in these solid portions the tendency to form narrow lumens was evident. The surrounding thyroid parenchyma was not invaded by tumor cells; its follicles were of small size. In the left lobe many small nodules were found which were composed of follicles, some of large dimensions. The lumens were filled with well stained colloid and the thyroid epithelium was of cuboid

FIG. 2. Cavernous blood vessels in hyaline layer. Hemorrhage surrounding vessels. ×70.

form. The histologic diagnosis was hemangioendothelioma of the right lobe and multinodular colloid goiter of the left lobe of the thyroid.

The postoperative course was uneventful and on August 5, 1951, the patient was dismissed from the hospital with a healed wound. In September 1952, she appeared to be in good health, without evidence of recurrence or metastasis.

REVIEW OF THE LITERATURE

History: Fifty-seven original case reports were available to us. In Langhans' institute, Limacher (5) observed the first 2 cases in 1898; in 1907, Frattin (6) described 2 cases, and Hedinger described 6 cases in 1909. Wegelin (7) observed 41 cases in thirty-one years. Under Wegelin's supervision, Finaly (8) described 6 cases in 1928 and Mollwo (9) 4 cases in 1947. The clinical features of this tumor have been summarized by DeQuervain (10), who saw 27 cases of hemangioendothelioma among 200 malignant goiters.

Gross pathology: Of the 57 cases in the literature, 24 had a solitary node. The size of these nodes varied between 1.5 cm. and 11 cm. in diameter, the average being 6.8 cm.; nine nodes were over 8 cm. in diameter. Two specimens had three nodes and five had two nodes each. Seven were multinodular goiters. Hemorrhagic cysts were present in 9 of DeQuervain's cases, and in 21 of the others. In cases in which several small tumor nodes occurred in place of a single one, it was not possible to exclude metastatic

growth in the thyroid tissue proper. Very often there were benign adenomas in the same thyroid. A tumor thrombus in the jugular vein was recognized grossly in Limacher's first case, and in 2 of Wegelin's cases. Metastases were not very common. In 6 of 15 autopsy cases reported in the literature, metastases were found in the lung. In 7 cases they were

FIG. 3. Fibrous capsule of tumor, not invaded by tumor cells. Compressed thyroid tissue at right. ×70.

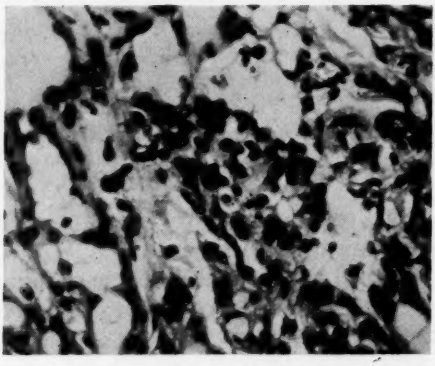

FIG. 4. Vascular area of tumor. Many spaces lined with atypical endothelial cells. ×270.

found in the pleura, causing hemothorax. Metastases in cervical or tracheobronchial lymph nodes were observed in 4 autopsy cases. Usui (11) found multiple metastases in the skull, in both tibiae, and in the right femur. Clivio's (12) patient had metastases in the sternum and humerus, in addition to a liver node which had caused hemoperitoneum. Wegelin observed metastases in lungs, pleura, liver, adrenals, heart and choroid plexus. Two of DeQuervain's patients had vertebral metastases.

Histology: According to Hedinger, the first stage of tumor development

is characterized by multiplication and widening of well defined blood vessels. In this stage, there is already a certain irregularity of the endothelial lining. From these capillaries a progressive proliferation of endothelial cells arises. The cells enlarge in size, the nuclei often become dark stained, and mitoses are numerous. The cytoplasm is eosinophilic in some cells and basophilic in others. Multinuclear, irregularly shaped masses of cytoplasm may form syncytium-like structures. Red blood cells may be engulfed by tumor cells and may form Russel bodies. Frequently the tumor cells form several layers around blood vessels or they may fill out the lumen. Such solid cell strands may be easily mistaken for carcinoma.

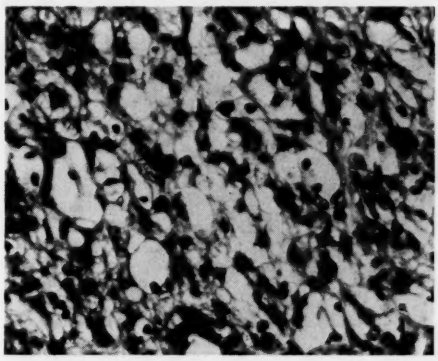

FIG. 5. Solid proliferation of endothelial cells protruding into lumen of vessel. Giant nuclei in some cells. Lattice structures in several vessels by invading tumor cells forming small spaces. ×270.

Incidence and histogenesis: Hemangioendothelioma of the thyroid is not uncommon in mountainous goiter regions. In Bern, the center of the Swiss goiter belt, 16.2 per cent of all malignant thyroid tumors are of this type (Matti). Wegelin observed 41 cases from 1907 to 1938, and DeQuervain observed 27 among 200 malignant goiters. The 7 cases described by French writers (13, 14) also came from mountainous goiter regions (Haute Savoy), and the 12 cases of Stühlinger (15) were from the Austrian Alps. No cases have been reported from the northern plains of Europe, and only 2 cases have been noted in North America. This discrepancy may be explained by the difference in structure between goiters in mountainous regions and in the lowlands. Comparative studies (16) have shown that in Switzerland 70 per cent of surgical goiters are fetal adenomas, against 3 per cent in our surgical material. Apparently the same factors which produce a high incidence of fetal adenoma are also responsible for the relative frequency of hemangioendothelioma in Switzerland.

Sex and age: Of the cases reported in the literature, 60 per cent were in

males and 40 per cent in females. The median age was 49.5 years; the youngest patient was 21 years of age and the oldest 71 years of age. Eight of DeQuervain's patients were over 60 years old.

Clinical findings: According to Hedinger, sudden and rapid increase in the size of a long-standing goiter is characteristic of hemangioendothelioma. Of the 57 reported cases, 2 had a goiter for sixty years, 9 for more than twenty years, and 1 for four years. In 6 cases the duration was from two weeks to one and a half years. Nineteen patients had a long-standing goiter and sudden increase in size brought them to their physicians for

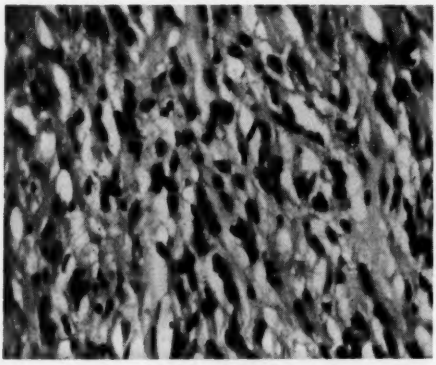

FIG. 6. Area resembling spindle cell sarcoma. Many cells show vacuoles. ×270.

consultation. The time interval between increase in size of the goiter and hospital admission varied between five weeks and one year. Bosse's (1) case was that of a 68-year-old man who had a cough and hemoptysis for five months, in addition to hoarseness and swelling of the right side of the neck. In DeQuervain's (10) experience, increase in hardness in one area was more important than rapid enlargement of a long-standing goiter. Decreased mobility of the goiter was regarded by him as the most important objective symptom. Radiating pain was noticed in 7 of DeQuervain's cases, but in only 4 of the other cases described in the literature. Two of DeQuervain's patients and 4 of the others had hoarseness, 3 patients had difficulty in swallowing, and 6 patients complained of marked loss of weight. Enlarged lymph nodes in the neck were noticed in 6 of DeQuervain's cases and in 2 of the others. Invasion of the trachea was present in 2 of Hedinger's cases, also in Winnen's (17), Usui's (11), and Simon's (18) cases, causing cough and bloody sputum. Simon's patient died on the seventh day after admission, from hemoptysis. The first manifestation of the disease in Clivio's second case was a spontaneous fracture of the right humerus due to metastasis. A cough and bloody sputum anteceded the

increase in size of a long-standing goiter in the case of Sapinski (19). The patient reported by Winnen had bloody sputum and a cough for two months, several weeks before his goiter became larger. Loss of weight and pain in the chest, due to pleural metastases causing hemothorax, was the initial symptom in Sapinski's second case.

Mosto and Sibilla (20) described the only case of hemangioendothelioma associated with symptoms of hyperthyroidism. The patient lost weight, had tremor, nervousness, and a basal metabolic rate of +30 per cent. Blum (22) described a fatal case of hemothorax due to metastatic hemangioendothelioma of the pleura. Matti (3) saw metastases in the oral mucosa.

Follow-up data are available in only 17 case reports. Without treatment, 7 patients died from a few hours to three and a half months after admission to the hospital. Of the surgical patients, 7 died from one day to four months after operation. Rice's (2) patient, who had an inoperable tumor on admission, received x-ray therapy following biopsy and was living and well two years later. The patient of Butureanu *et al.* (13), who received combined treatment, died four months after the onset of the disease.

SUMMARY

A case of hemangioendothelioma of the thyroid has been observed in a 67-year-old female patient. It is the third case described in North American patients.

The rarity of the disease in this country, in contrast to its relative frequency in Switzerland, is noteworthy. It can be explained by the relative rarity of fetal adenomas in this country. Hemorrhage into a fetal adenoma with formation of "rubber hyaline" predisposes to the development of hemangioendothelioma.

Acknowledgment

Dr. Wegelin of Bern reviewed the microscopic sections of our cases. He regarded the tumor as a hemangioendothelioma and felt that, because of its definite encapsulation, the tumor was probably not very malignant; however, he would not exclude the possibility of metastases. Dr. A. Crotti and Dr. A. Graham concurred in the histologic diagnosis of hemangioendothelioma.

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Letter to the Editor

LOCAL AND SYSTEMIC USE OF ETHINYL ESTRADIOL IN PATIENTS WITH ACNE AND HIRSUTISM

To the Editor:

The local application of estrogen in the management of acne was first reported by Simons in 1938 (1). Since then, multiple and frequently contradictory reports have appeared in the literature (2–8). In all instances so far reported, the estrogen has been used in an ointment or lotion base. Frequently the concentration of estrogen has been relatively low. In a fairly large series of patients, one of the authors (L.K.) in collaboration with Drs. Julian Lunsford and Herman Allington used an estrogen ointment. None of us was impressed by the results obtained. A number of patients complained of exacerbation of the acne. This was considered to be referable to blockage of the ducts of the sebaceous glands by the light ointment base which was used.

For the reasons to be noted below, during the past five years two groups of patients, 47 whose major complaint was hirsutism and 38 who had acne of severe or of moderately severe degree, have been evaluated from the standpoint of effect of local and systemic estrogen. All of the patients with acne had previously received nonendocrine therapy. The majority were referred by dermatologists because of inadequate response to such therapy.

The local estrogen used was an alcoholic solution of ethinyl estradiol in concentrations varying from 1 to 100 mg. per 100 cc. of 70 per cent ethyl alcohol or isopropyl alcohol. An adequate concentration seemed to be 5 mg. per 100 cc. Patients were instructed to apply the material to all involved areas, patting it on with the finger tips. After initial application the material was rubbed in thoroughly for a period of five minutes. This procedure was usually carried out twice daily.

Systemic ethinyl estradiol was administered in a dosage of 0.05 to 0.1 mg. daily in most instances. Only female patients have received systemic estrogen, as it is still undetermined whether permanent testicular damage may result from prolonged therapy.

RESULTS

Acne

Significant improvement occurred in all patients treated. Of the 38,

29 required both local and systemic therapy. Nine responded adequately to local therapy alone; none of these had "cystic acne."

Typical Case Reports

Female patient HOV, aged 31, had a history of severe cystic acne dating from the appearance of puberty at the age of 13. Estrogen therapy was begun in August, 1948. Local application of an estrogen-containing ointment for a period of two months resulted in significant exacerbation of the acne, presumably referable to the ointment base. Local application of an alcoholic solution containing 20 mg. of ethinyl estradiol per 100 cc. to one side of the face, at the same time that a simple alcoholic solution was applied to the other side of the face, resulted in significant improvement on the treated side but essentially no change on the control side. Active acneiform lesions, however, were still present on the estrogen-treated side after four months of therapy. The patient then received a daily dose of 0.05 mg. of ethinyl estradiol orally, and subsequently 0.1 mg. daily, with discontinuance of such oral administration during the first five days of each month to permit of withdrawal bleeding. Within three months almost complete disappearance of acne was noted. After six months of treatment, medication was discontinued and there was recurrence of severe acne. Resumption of oral therapy again resulted in subsidence of the acne. Subsequently local therapy was resumed and systemic therapy was gradually discontinued. The patient was adequately maintained on local therapy for approximately six months, at which time she became pregnant.

Four months after the birth of her child, she was seen again with a recurrence of severe cystic acne—all therapy having been discontinued for a period of several months. Resumption of systemic estrogen administration resulted in nearly complete subsidence of her acne within a period of three weeks.

Male patient FRO, aged 19, suffering from rheumatoid arthritis, experienced severe acne in association with combined ACTH, cortisone and testosterone therapy. Local application of an alcoholic solution containing 4 mg. of ethinyl estradiol per 100 cc. resulted in nearly complete control of the process. Improvement was noted within ten days after the local therapy was begun, and was maintained.

Hirsutism

Of the 47 patients suffering from facial hirsutism who were treated (22 for a period of more than three years), 8 showed significant improvement, 19 showed possible improvement, and 20 showed no change. These patients will be followed for a period of ten or more years, inasmuch as it is felt that hirsutism, once well established, will be resistant to therapy of any sort (see following case history), and consequently improvement in most instances will be noted only after prolonged therapy. Without exception, the patients who showed significant improvement were those with moderate degrees of hirsutism of a duration not in excess of five years.

Female patient IGN, was 28 years old and had hirsutism of more than fourteen years' duration, resulting from an adrenal tumor which had been removed more than four years previously. This patient has been reported elsewhere (9). Despite the removal of the exciting cause of the hirsutism, only a slight decrease in the rate of hair growth

occurred. This decrease appeared to have reached a plateau by the end of one year following operation. The application of ethinyl estradiol solution for a period of nearly two years produced little or no change in the hirsutism. The observation in this patient is cited to suggest that, even though the exciting cause of excessive hair growth may be removed, hair follicles once stimulated tend to remain functionally active.

DISCUSSION

Acne and hirsutism have certain things in common:

1. They are most apt to appear at, or following puberty.
2. Both are prone to occur during treatment with androgens, with corticotropin, or with cortisone-like steroids.

The majority of patients with spontaneous acne or hirsutism have no evidence of real endocrine abnormality. It seems proper, therefore, to divide the conditions into two general types:

1. Endocrine, *i.e.*, referable to the effects of excessive amounts of androgen or other steroids, as in the case of adrenal or gonadal tumors, or adrenal hyperplasia.
2. End-organ, *i.e.*, referable to excessive sensitivity on the part of hair follicles and sebaceous glands to the effects of normal amounts of androgens and certain cortical steroids.

Since it is well established that estrogens are antagonistic in a number of respects to hormones having androgenic effects, it seems feasible to

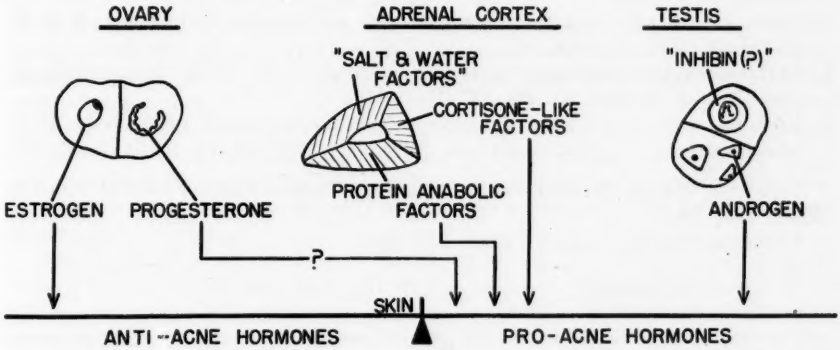

FIG. 1. The relationship of steroid hormones to acne and hirsutism.

assume that in patients with acne and hirsutism the skin and its appendages respond in opposite fashion to these hormonal factors. This concept is shown diagrammatically in Figure 1.

Acknowledgments

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OBSCURE INTRAUTERINE LESION AS CAUSE OF MENORRHAGIA

It is interesting to note the paucity of cases of menorrhagia in which this symptom is actually due to a hormonal abnormality usually described as some kind of dysfunction of the pituitary-ovarian-endometrial axis. The following case report dramatically emphasizes the necessity of thorough and sometimes radical investigation before a patient is placed in the category of what is variously called functional menorrhagia, dysfunctional menorrhagia, metropathia hemorrhagica or menorrhagia due to abnormal endocrine function.

CASE REPORT

S.S., a 24-year-old single, white female was first seen in January 1952 with the complaint that for three years there had been a gradual increase of bleeding at the time of menstruation. Menstruation had begun at 12, and a cycle of twenty-eight to thirty days had been fairly constant throughout her menstrual life. Until three years previously, the periods had been normal in amount, requiring about three to four pads per day, and lasting for about five or six days. There had been fairly severe menstrual cramps and slight premenstrual mastalgia. During the preceding three years, however, the periods had gradually increased in amount and duration until, at the time the patient was seen, they lasted eight to nine days and were so profuse that she rapidly soaked through two perineal pads and it was necessary for her to stay at home for the first two or three days of her period. She had received various types and varieties of what was thought to have been "hormone medicine" for over a year but it had no effect on her menstruation.

Physical examination disclosed an anemic appearing, but otherwise normal young woman. Breast development was normal and pelvic examination disclosed a small nulliparous cervix with a small uterus, which was normal in position and motility. No abnormalities were felt in the adnexal region. Laboratory investigation at this time showed the following blood picture: hemoglobin 8.6 Gm., and RBC 3,370,000 with slight anisocytosis and poikilocytosis; platelets were 205,000 and appeared normal on the smear. Prothrombin time was 17.9 seconds, which was within normal range, and the bleeding and clotting times were normal. Serologic examination and urinalysis gave negative results. A premenstrual curettage was carried out and no abnormalities were noted within the uterine cavity. The curettings were described as diagnostic of "late secretory endometrium." The curettage did not improve the patient's subsequent menstruation and she was then started on methyltestosterone therapy, 10 mg. per day for the first twenty-five days of the menstrual cycle. She was also given small doses of ergotrate at the time of menstruation. A hysterogram was suggested at this time but

for various reasons was not carried out until after two months of testosterone therapy, which proved to be entirely unsuccessful. The menorrhagia had by this time become so profuse that her periods extended over a period of about two weeks. In order to stop the bleeding so that a hysterogram could be carried out, the patient was given 150 mg. of stilbestrol within about four hours and following the styptic effect of this medication, a hysterogram was obtained under fluoroscopic control (Fig. 1). The hysterogram dis-

FIG. 1. Partially lateral hysterogram view of the uterine cavity. The superior arrow points to the filling defect on the posterior wall, as described in the text. The inferior arrow points to the large and partially dilated cervical canal which resulted from the previous large estrogen dosage.

closed a definite filling defect on the posterior wall of the uterine cavity. The lesion was thought to be a submucous fibroid; even though it was not particularly large, it was assumed to be the probable cause of the continued profuse bleeding, and a myomectomy was advised. At operation a preliminary dilation and curettage was carried out and a small amount of normal appearing endometrium was obtained. Although special at-

tempts were made to identify an abnormality on the posterior wall of the uterus by means of the curette, no irregularity could be identified. A laparotomy was therefore performed. Upon opening the peritoneal cavity the uterus was found to be normal in size and position, and both inspection and palpation of the uterus disclosed no irregularities in the shape or consistency which could be interpreted as a submucous or intramural fibroid. Both ovaries appeared to be normal and no local pathologic condition was observed. A decision was then made to open the uterus and observe the uterine cavity by direct inspection. A tourniquet was therefore placed around the lower uterine segment and the uterus incised anteroposteriorly from the fundus to the level of the internal os. Inspection and palpation of the surface of the uterine cavity exposed to view in this manner disclosed an irregular, firm, granular-feeling discrete area about 2 cm. in diameter,

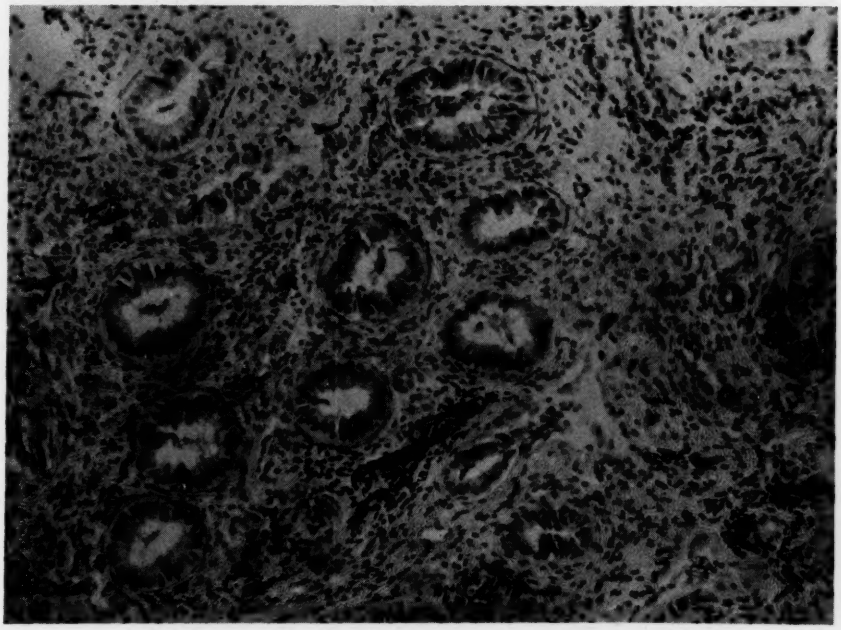

FIG. 2. A low-power photomicrograph of the lesion, diagnosed as adenomyosis, which was removed from the posterior surface of the endometrial cavity.

which was raised about 2 or 3 mm. above the curetted surface of the posterior wall of the uterus. This area did not have the appearance or feel of a submucous fibroid and its consistency and irregularity suggested the diagnosis of adenomyosis, which it subsequently proved to be (Fig. 2). The lesion was excised without difficulty, the incision in the uterus repaired with two layers of 00 chromic catgut, and the serosal surface closed with a Lembert suture of 000 plain catgut. Following removal of the appendix the abdomen was closed in the routine fashion. Subsequent to the operation the patient has had three menstrual periods at intervals of from twenty-eight to thirty days. The periods now last for four days, require two to four pads a day, and are associated with moderate

menstrual cramps. A biopsy before the second postoperative menstrual period disclosed normal secretory endometrium (Fig. 3).

DISCUSSION

This case is thought to be of considerable significance for several reasons. If we are to assume that the bleeding was caused by the submucous adenoma it is of interest to note that this lesion was identified only at the time of the hysterogram and could not be felt with the curette at the time of curettage, even though special attempts were made on two occasions

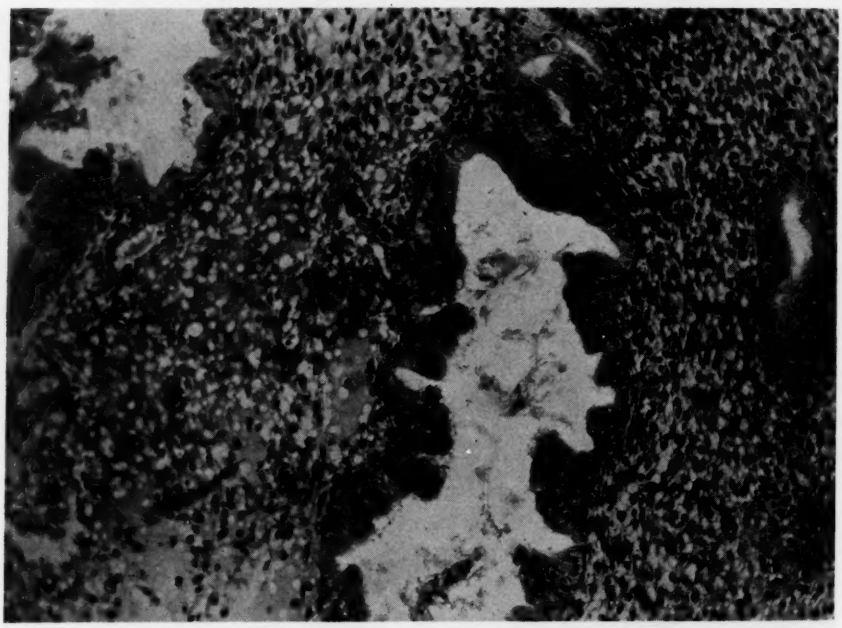

FIG. 3. Low-power photomicrograph of premenstrual biopsy specimen, showing secretory endometrium. The specimen was taken before the second postoperative menstrual period.

to identify some endometrial abnormality or irregularity. Furthermore, it is even more impressive that when the uterus was actually observed and palpated at the time of laporotomy, no irregularity could be either seen or felt on, or within, its surface. It was therefore necessary to open the uterine cavity before the lesion was identified. It is impossible, of course, to say positively that this area of adenomyosis was the cause of the menorrhagia, but such an assumption would be fairly justified since, following excision of the area, the patient has had no further difficulties with menstruation. This case provides further evidence for the usefulness

of hysterography in the diagnosis of intrauterine lesions and also provides the rather unusual instance of a situation in which the uterine cavity had to be opened before the lesion was identified.

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Editorial

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COMMENTS ON THE EXOPHTHALMOS OF GRAVES' DISEASE*

GRAVES' disease is a systemic disorder of unknown origin which is associated with diffuse hyperplasia of the thyroid gland, and usually with hyperthyroidism, an easily detected goiter and certain typical eye signs. Either the hyperthyroidism or the eye signs may occur alone. In recent years much attention has been given to patients with exophthalmos who show little or no evidence of hyperthyroidism. This has served to emphasize the impression that in clinical Graves' disease the onset of hypometabolism or hypothyroidism aggravates eye changes, if they are present. Clinical or experimental hypothyroidism may be undesirable if exophthalmos is present; nevertheless, it should not be overlooked that exophthalmos may become extremely severe in the presence of active hyperthyroidism. Recorded cases (especially before the development of modern thyroid surgery) show instances of loss of eyes from ophthalmitis arising from exophthalmos during active hyperthyroidism, when surgery was not performed and nothing was done which might have effectively controlled the hyperthyroidism (1–3).

Exophthalmos, as clearly indicated by the name, means proptosis. However, the term is commonly used to designate the complex eye signs associated with Graves' disease. An appearance of fullness of the lateral portion of the upper lids may be early evidence of such eye changes but

* From notes used at a symposium on thyroid diseases for the New York State Medical Association, New York City, May 15, 1952.

the commonest early sign is lid-lag. This is the inability of the upper lid to follow the movement of the eye, as the eye follows the examiner's finger upward and downward at varying speeds, at a distance of two feet. Lid-lag precedes the state of constant retraction of the upper lids.

Retraction of the upper lid may produce an appearance of exophthalmos on one or both sides before proptosis is actually present. As measured by the exophthalmometer, there may be an increase from the low normal level of 14 mm. in the A.P. position of the bulb, to the high normal level of 23 mm., before exophthalmos can be proved by one measurement. This fact points to one serious inherent weakness in depending upon such measurements, unless repeated observations indicate a trend. However, a single measurement of 22 mm. may be of considerable importance if the measurement of the other eye is 4–5 mm. or less.

Other signs which may be closely related include conjunctival edema, lacrimation, conjunctival chemosis, bulging of the lids, resistance to posterior displacement of the bulb, palsy of the superior rectus, palsy of the other extra-ocular muscles, lid edema, choked disc, corneal ulcer and ophthalmitis. On the basis of such associated eye changes, many signs have been described which relate to this type of exophthalmos.

The natural history of exophthalmos is not well understood but there are a few striking facts. The progress of exophthalmos may vary a great deal and this has served to confuse investigators with regard to possible aggravating factors and the possible benefits of various types of treatment. Some of the variations may be enumerated as follows: Exophthalmos may occur and become extremely severe in a few weeks. It may appear for two or three weeks and recede as quickly. Most cases of severe progressive so-called malignant exophthalmos occur in patients over 30, and usually over 40 years of age. Over 65 per cent of cases of malignant exophthalmos occur in men, which is in decided contrast to the sex distribution of Graves' disease. Proptosis may increase 1 mm. every one or two months for a year or two, maintain a plateau for a similar period of time, and then gradually recede. Such a progressive increase in proptosis may even follow wide orbital decompression, and recession may not begin until a year or more after the operation.

There is no close relationship between hyperthyroidism and exophthalmos. Exophthalmos may appear years before hyperthyroidism or years after its control and without its recurrence. When severe exophthalmos develops in the presence of little or no hyperthyroidism, it has been referred to as exophthalmic ophthalmoplegia or hyperophthalmopathic Graves' disease. The opposite situation is more common, namely, severe hyperthyroidism without eye signs.

PATHOLOGY

The orbital changes are chiefly those of deposition of fat, white blood cells, and fibrotic tissue (4). With respect to the levator palpebrae, however, the factor of sympathetic nervous stimulation is more apparent than in connection with the other ocular changes. A cervical sympathetic nerve block in a patient with pronounced lid retraction will cause a lowering of the lid associated with the production of Horner's syndrome, just as ptosis is associated with this change in normal persons.

The retrobulbar tissue is increased in volume. There is an apparent increase in the actual amount of fat, as the autopsy studies of Rundle and Pochin (5) on 17 patients with severe exophthalmos showed. Apparently an added edema is a variable but important factor. Cellular infiltration and finally deposition of fibrous tissue occur in this fat. The bulging of the lids in a lumpy fashion may be due to the fact that they are pushed forward by fat protruding between the ocular muscles and over the orbital brim, where it can get out most readily. Below the eye such irregular bulges may be seen laterally, and less so medially; above the eye they are primarily lateral. In other cases the lids appear to be involved in watery edema. At times the conjunctiva becomes so edematous that it falls over the cheeks in huge sacs. The fact that setons placed through such sacs do not cause a disappearance of the watery-looking swelling indicates it is not the same as other types of edema. The possibility of its being hyaluronic acid or related material needs further investigation. The work of Asboe-Hansen, Iversen, and Wichmann (6), of Ludwig, Boas and Soffer (7) and others indicates that mucopolysaccharides are intimately involved and their great avidity for water may be closely associated with the tendency to edema which is present.

The extra-ocular muscles may be enlarged to several times their normal size. They are edematous, infiltrated with lymphocytes and fat, and finally may become densely fibrotic. Rundle and Pochin (5) found that the fat content of the levator palpebrae superioris was higher than that of the other muscles.

ETIOLOGY UNKNOWN

Theories of etiology which invoke such causes as sympathetic stimulation, contraction of Müller's muscle and venous congestion have been rightfully discarded.

Smelser (8) in 1937 showed that changes in orbital structures similar to those seen in clinical exophthalmos may occur in animals injected with pituitary extracts which contain thyrotropic hormone. Evidence that the ocular changes are due to thyrotropic hormone is circumstantial. One of the greatest obstacles to a logical answer to this question, is that pure

thyrotropic hormone has not been available. It is interesting, however, that inactivation of thyrotropic hormone has been shown to be associated with the loss of exophthalmos-producing power (9).

The most popular current theory is that the exophthalmos of Graves' disease is caused by thyrotropic hormone in the human. Such a thesis is on an even more unstable basis than its experimental counterpart and is far from confirmed. Until it is, such terms as "pituitarogenic exophthalmos" would seem to be totally unwarranted.

Reasons for believing that human exophthalmos may be due to pituitary hormone, possibly thyrotropin, are as follows:

1. Exophthalmos is produced in animals by pituitary extracts which contain thyrotropin.
2. There are claims of improvement following roentgen-ray therapy to the pituitary region in human beings (10).
3. Anterior pituitary damage has been caused by cautery, in human beings who had arterial hypertension and who also had exophthalmos of Graves' disease. Upon decompression of one orbit, the proptosis of the opposite eye receded several millimeters (11).
4. Exophthalmos develops in some rats given thiourea; and since excess production of thyrotropin is known to take place under such circumstances, thyrotropin has been suspected to be the cause of the exophthalmos.

Reasons for believing exophthalmos may be of pituitary but not of thyrotropic origin are:

1. Exophthalmos is produced by injection of pituitary extracts into animals which have been thyroidectomized. Since there is no target-organ present, the effect under these conditions could scarcely be said to be a thyrotropic effect.
2. Crude pituitary extracts tend to cause a good deal of exophthalmos, whereas those purified for thyrotropic effect may produce very little eye change (12).
3. Thyroid feeding does not cause exophthalmos in humans. If thyrotropic hormone caused exophthalmos in the human, it might be argued that this effect would be producible by thyroid feeding, *since production of thyroid hormone* is the logical effect of pituitary thyrotropin. The exophthalmos of fish has been thought to be due to thyroid feeding. In some instances it may be due to intra-orbital edema from other causes (13).

Reasons for doubting that pituitary thyrotropin or other pituitary hormones cause the exophthalmos of Graves' disease:

1. Exophthalmos of the type seen in Graves' disease has not been

reported in any instances of pituitary tumor. A single example of exophthalmos of this type in a patient with a pituitary neoplasm would strengthen the thesis of pituitary origin of exophthalmos. Incidentally, there is no evidence which seems convincing that hyperthyroidism is ever caused by hyperactivity of the pituitary associated with a pituitary tumor.

2. If it is a fact that thyrotropic hormone causes both the hyperthyroidism and the exophthalmos of Graves' disease it might be expected that some eye signs would be found in almost all patients, especially if the disease is severe or of long standing; but such is not the case.
3. If thyrotropin causes human exophthalmos, an excess of thyroid hormone might be expected under some circumstances to inhibit the pituitary and prevent the progress of eye signs. This inhibition does not occur in Graves' disease, and there are no reports of success with thyroid therapy in patients with progressive eye signs and normal or low metabolic rates.
4. Exophthalmos does not appear consistently in treated patients with Graves' disease who have measurable thyrotropic hormone in the urine; such studies have been painstakingly made by Purves and Griesbach (14).
5. Also, according to these workers, patients with malignant exophthalmos may or may not have a high level of serum thyrotropic hormone. The Danish workers (6), however, have produced evidence to show that in the cases they studied, "thyrotropin was significantly increased in nine out of ten cases of progressive exophthalmos."
6. Exophthalmos does not appear in patients with spontaneous myxedema, even though they may have high blood levels of thyrotropic hormone. It is seen only rarely in persons with true myxedema of postoperative origin. It is true that patients with severe and progressive exophthalmos may have basal metabolic rates below −20 per cent, yet a careful clinical evaluation will not show that they have myxedema.
7. In patients with exophthalmos, moderately large doses of roentgen irradiation to the pituitary have been shown to lower sperm counts decidedly. This would seem good evidence that the pituitary has been suppressed. However, such lowering is maintained for only about two months (11); the sperm count probably rises because the pituitary gland has recovered its ability to produce gonadotropin. It seems reasonable to assume that the pituitary under such circumstances also recovers the ability to produce thyrotropin. This finding raises the question of the validity of the conclusion that the improve-

ment in proptosis sometimes reported following roentgen-ray therapy to the pituitary is actually due to the treatment given (10).

TREATMENT

1. The reason that control of hyperthyroidism by antithyroid drugs, by I^{131}, or by surgery usually leads to remission of all signs of Graves' disease is unknown. It is common for exophthalmos to increase slightly after thyroidectomy, but in most patients this increase is of no practical importance and in a matter of months in most persons there is a trend toward normal, though some remaining changes are the rule.

2. The use of thyroid feeding in the management of exophthalmos is of no proved value. However, since there is sufficient suggestion from experimental sources that hypothyroidism may cause aggravation of exophthalmos, it is prudent to avoid hypothyroidism and give thyroid if necessary for this purpose.

3. There is some evidence that iodine causes an inactivation of thyrotropin (15). On this basis, small doses of iodine may be tried.

4. Because estrogens are the best available pituitary inhibitors, they have been tried in the treatment of exophthalmos but no value has been proved.

5. Rest, physical and mental, and sedation are also usually considered advisable but are not proved to be of actual value.

6. If the patient sleeps with the head high, some of the edema of the lids and orbit may be prevented. Sodium restriction may be tried.

7. In severe cases the cornea should be protected from drying, by ointments and goggles.

8. Roentgen-ray treatments to the pituitary have been advocated. The effects of such therapy on a nontumorous pituitary are probably transient and the results, both ours and those reported by others, fail to convince us of their value.

9. Operative treatment for exophthalmos includes:

(a) Tarsorrhaphy.
(b) Seton drainage of invasive conjunctival swelling.
(c) Lid suture (usually reserved for those patients in whom loss of an eye appears inevitable without it).
(d) Orbital decompression.
(e) Extra-ocular muscle surgery. This may be of value in some cases in which the process has become quiescent and in which extra-ocular muscle palsy causes persistent diplopia.

Other modes of treatment of the exophthalmos of Graves' disease are symptomatic or are largely theoretic and of unproved clinical value.

The foregoing notes emphasize the fact that the cause of Graves' disease and that of the associated exophthalmos are unknown. Exophthalmos fortunately usually recedes following a remission of Graves' disease. Why this regression occurs so frequently following an attack on the target organs, without an attack on the cause of the disorder, is an unanswered question and an important part of this unsolved problem.

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Questions and Answers

(Questions submitted at the 1952 Postgraduate Assembly of the Endocrine Society)

Answers by Richard Meyer, Chicago, Ill.

QUESTION: How long after giving ACTH or cortisone can tests for adrenal insufficiency be performed and authentic results obtained?

ANSWER: It is assumed that the inquiry relates to patients with essentially normal adrenal cortical function *prior* to initiation of ACTH or cortisone therapy, *e.g.*, in rheumatoid arthritis.

Suppression of adrenal cortical function following moderately intensive or prolonged cortisone therapy has been shown by Sprague *et al.* (Proc. 2nd Clin. ACTH Conf. Blakiston Co., Philadelphia, Pa., Vol. II, p. 38, 1951), to last for two to three weeks. The four-hour eosinopenic response to a standard 25-unit dose of ACTH intramuscularly is usually impaired during this post-cortisone period. Experience has shown that the adrenal cortex will recover completely after this time or much more rapidly with the aid of intensive ACTH stimulation (two to three days); irreversible damage to the cortex has not been observed (Engleman *et al.: Arch. Int. Med.* **91**: 1, 1953). Following ACTH therapy a period of relative adrenal insufficiency may occur which is usually of short duration (twenty-four to forty-eight hours). Tests for adrenal insufficiency will be valid after the adrenal cortex has recovered in respect to the above time relationships.

QUESTION: Why do eosinophils decrease in the blood following removal of both adrenals?

ANSWER: Following removal of both adrenals in untreated animals, changes in the eosinophils have been inconsistent. Eosinopenia has been observed but usually the eosinophils remain stable or actually increase. It is of interest that patients with Addison's disease undergoing major surgery fail to show the postoperative eosinopenia so characteristically seen in patients with normal adrenal cortical function submitted to surgical stress (Roche *et al.: J. Clin. Endocrinol.* **9**: 662, 1949). Eosinopenic response to stress of various kinds can be restored in adrenalectomized animals by the administration of cortisone or adrenal cortical extract. The eosinopenia observed is apparently due to a "peripheral synergism" with some product of stress, such as epinephrine with cortisone or compound F.

The experience of Huggins and Bergenstal at the University of Chicago in patients undergoing bilateral adrenalectomy for prostatic carcinoma is in line with what might be expected from the experimental data given. They have observed eosinopenia for several days after the removal of both adrenals. All patients are pretreated with large doses of cortisone prior to, during, and following surgery. When maintenance dosage of cortisone is established (after the first week) the eosinophils in the blood return to preoperative levels in most patients, but in some the eosinophils may rise to levels exceeding those found preoperatively.

Answers by Ray F. Farquharson, Toronto, Canada

QUESTION: Has cortisone therapy helped patients with panhypopituitarism?

ANSWER: Cortisone is of great value in the treatment of patients with severe anterior pituitary insufficiency. Since adrenal cortical insufficiency is the most important and serious manifestation of this condition, cortisone should be given first place in its treatment not only in acute crises (intravenously if necessary) but also in chronic phases of the condition (25 to 50 mg. per day in divided doses with added salt and, if necessary, desoxycorticosterone). The associated underfunction of the thyroid and gonads may be treated later.

QUESTION: What is the effect of prolonged starvation (Ancel Keys' experiments) on the pituitary, gonads and other glands?

ANSWER: Experimental studies of prolonged caloric undernutrition in animals demonstrate no constant changes in the anterior lobe of the pituitary gland other than a general shrinkage in size of cells and a tendency to lose specific staining reactions so that the number of chromophilic cells is reduced. In extreme starvation there may be severe degenerative changes with edema and congestion, such as occur in other organs (1). The changes in the gonads are more marked. The ovaries show retardation of development of follicles, followed by atrophy with resultant sterility. There is similar atrophy and underfunction of the testes, with shrinkage of the prostate gland and seminal vesicles. It is often postulated that these changes are due to reduced secretion of gonadotropin by the pituitary gland, although the gonadotropin content of the gland per unit of tissue has been found to be normal or increased. The changes in the gonads in starvation have been reversed by injection of pituitary or chorionic gonadotropin. The thyroid and the adrenal glands are affected to a lesser extent, there being considerable species variation in the adrenal gland especially. The effects of moderate undernutrition appear to be reversible (2); in extreme starvation focal areas of degeneration may persist.

The Minnesota experiment (Ancel Keys (3)) dealt with previously healthy young men who underwent voluntary semi-starvation (approximately 1,500 calories per day) for twenty-four weeks, while they continued in moderate activity; they lost about 25 per cent of their initial body weight. In these subjects, the basal metabolic rate decreased by about 38 per cent. The excretion of 17-ketosteroids in the urine was reduced to about 8 mg. per twenty-four hours. The sperm counts were not reduced but motility of the sperm and the duration of motility were affected. The fasting blood sugar level fell to between 60 and 65 mg. per 100 cc. (It is interesting that Wrenshall has found the insulin content of the pancreas of emaciated subjects, calculated per kg. of body weight, to be decreased.)

In pathologic observations on human emaciated subjects there is little to suggest that the endocrine glands are more affected than other tissues, such as the heart and liver. The loss in weight of the pituitary gland appears to be proportionately less than that of the whole body. The histologic changes are similar to those found in other tissues, there being in severe emaciation a decrease in the number of chromophilic granules, vacuolation of cytoplasm, and nuclear degeneration and congestion. Similar, perhaps less marked changes are found in the thyroid and adrenal glands. In the testes, an increase in the interstitial cells is commonly observed, associated with a maturation arrest of spermatogenesis; in severe cases there may be hyalinization and atrophy of the seminiferous tubules (5). Ovaries show decreased follicular development.

It is natural that the functional changes should be less marked than when there is a diffuse local lesion of an endocrine gland. Decreased excretion of FSH has been described (4). The low BMR is not necessarily due to underfunction of the thyroid gland; in some cases of anorexia with low BMR the radioiodine uptake by the thyroid and the serum protein-precipitable iodine concentration have been normal. Amenorrhea is the rule in moderate to severe starvation, and in men sexual interest and desire disappear.

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Question: Is there any report in the literature of the appearance of severe persistent gastrointestinal disturbances (diarrhea) following destruction of the anterior pituitary postoperatively or by tumor pressure?

Answer: The occurrence of severe persistent gastrointestinal disturbance (diarrhea) following destruction of the anterior lobe of the pituitary gland at operation or as a result of tumor pressure must be very rare, if it occurs at all.

Answers by Edward Henderson, Bloomfield, N. J.

Question: Is methylandrostenediol of any value in the treatment of osteoporosis?

Answer: Although there are no reports on the treatment of osteoporosis dealing specifically with the use of methylandrostenediol, we may assume from metabolic studies on this compound that it is 1) a feeble androgen and 2) a feeble anabolic agent. In other words, in order to achieve the anabolic effect desired to treat osteoporosis properly, it would be necessary to give a dosage of methylandrostenediol that would very likely result in virilizing symptoms. This being the case, it is believed that oral methyltestosterone therapy would be preferable.

Question: What course would you pursue if the endocrine treatment of senile osteoporosis brought on uterine bleeding in a patient 80 years of age?

Answer: When the administration of estrogen has caused uterine bleeding in a patient of this age, two things can be done to obviate this undesirable reaction: 1) withdraw the estrogen altogether and administer methyltestosterone in a dosage of 10 mg. orally twice daily, or 2) reduce the daily dose of estrogen and add methyltestosterone. Tablets of a combination containing 5 mg. of methyltestosterone plus 0.02 mg. of ethinyl estradiol are available. The dosage would be one tablet daily.

Answer by John E. Howard, Baltimore, Md.

QUESTION: Should all patients with hypertension be screened for pheochromocytoma with a drug like Regitine, since it has been indicated that it is a comparatively safe agent?

ANSWER: It is my belief that, in the present state of our knowledge, all patients with the essential type of hypertension should be screened with a test of this kind. My preference is the benzodioxane test, perhaps because of far greater experience with it than with other drugs. It should be remembered, however, that there are on record several cases of proven (by removal and cure) pheochromocytomas in which the results of benzodioxane tests were negative. Contrariwise, in the presence of uremia, drugs of this type have induced sharp falls of blood pressure (false positive reactions) in persons who have later had a postmortem examination and in whom no medullary tumor was found. The clinical picture as a whole must be evaluated in making the decision whether or not to explore for a pheochromocytoma.

Answer by Danely P. Slaughter, Chicago, Ill.

QUESTION: What is the basis of the proposed operation to remove the pituitary, for temporary relief, in cases of far-advanced cancer?

ANSWER: It has been postulated that the adrenal glands, the gonads, and possibly the growth factor in pituitary secretion may have some effect on the growth potential of malignant tissue. The removal of the gonadotropic as well as the adrenocorticotropic and growth factor hormones could conceivably influence the rate of growth favorably in certain cases of cancer.

There is no experimental or clinical evidence to support the assumption that the use of female sex hormones in the normal patient will cause cancer. However, a word of caution should be included with this statement. Patients who have breast cancer may well be unfavorably influenced through the use of such medication. A similar statement might apply to patients with corpus cancer, in that the bleeding in these patients might be ascribed to the medication they were receiving and proper investigation and evaluation therefore delayed.

Answers by Robert B. Greenblatt, Augusta, Ga.

QUESTION: Please comment on the treatment of dysmenorrhea due to endometriosis in young women and in nullipara.

ANSWER: In a young nulliparous woman, dysmenorrhea resulting from endometriosis may be treated conservatively during the early reproductive years. There are several methods of therapy that yield moderately good, though temporary, results. If surgery is employed, removal of endometrioma with conservation of as much of the reproductive organs as possible is the goal, along with presacral nerve resection, suspension of the uterus and dilation of the cervical canal. In most instances an eventual second operation may be expected. If hormonal management is attempted, the following methods may be tried:

1. Androgen therapy frequently yields remarkably good results. A dose of 25 mg. of testosterone propionate given at weekly intervals, or 10 mg. of methyltestosterone in linguet form given daily during the intermenstruum may be employed for periods of five to six months, followed by a two-month or three-month interval during which therapy is

omitted. The implantation of two or three pellets of testosterone (75 mg. each) at six-month to eight-month intervals is the method of choice in our clinic. The disadvantages of androgen therapy, however, are manifold. It should be discontinued if and when more than a mild degree of hypertrichosis develops, or when the pitch of the voice is so lowered as to cause hoarseness and soreness of the vocal cords. The increase in libido which accompanies androgen therapy may be a welcome relief from marital drudgery in the wedded woman. On the other hand, it may prove most annoying and frustrating to the young unmarried woman and sedation may be necessary. The advantages of androgen therapy in the dosages outlined are that ovulation is not interfered with, dysmenorrhea and dyspareunia in a great measure are alleviated, and the hypermenorrhea usually present in endometriosis is lessened considerably.

2. Estrogen therapy. (a) Estrogens in small doses frequently aggravate the signs and symptoms of endometriosis. However, if estrogens are administered in large enough dosage to inhibit ovulation, relief will be obtained in a goodly number of patients. The method is as follows: Immediately after a menstrual period is over, the patient is given an injection of 10,000 r.u. of estradiol benzoate for two or three consecutive days. She is then immediately given oral estrone sulfate (1.25 mg., or its equivalent) three times daily. The estrogen is administered in this dosage continuously for the next six to twelve months. A withdrawal bleeding period is induced each month by the oral administration of anhydrohydroxyprogesterone, 10 mg. t.i.d. for five consecutive days. It must be emphasized that the estrogen administration is continued during the progesterone therapy as well as during the bleeding periods.

(b) For those patients in whom the foregoing method of treatment does not afford sufficient relief, the next step is the inhibition of menstruation by a gradually increasing dosage of estrogen. For economic reasons, stilbestrol or ethinyl estradiol may be preferred for most patients. The patient is given oral stilbestrol (1 mg. t.i.d., or its equivalent) starting on day five of the menstrual cycle, and the dosage is gradually increased until 5 mg. per day is administered. If and when any break-through bleeding occurs, it may be arrested by increasing the stilbestrol dosage by 5 mg. per day, and maintaining it at this new level. The patient may ultimately need to take 25 mg. of stilbestrol per day. It is advisable that therapy be continued for a year. When treatment is discontinued, 25 mg. of progesterone subcutaneously should be given for three consecutive days to bring on a good withdrawal bleeding period.

QUESTION: Are there any contraindications to cyclic estrogen-androgen therapy in healthy females 50 to 60 years of age, when it is insisted upon by patients themselves?

ANSWER: There are no contraindications to cyclic estrogen-androgen therapy in healthy females 50 to 60 years of age, if the physician deems such treatment advisable. The long continued use of this preparation will, in a certain number of patients, cause some facial hypertrichosis and voice changes. If these changes take place, the androgen therapy should be discontinued.

QUESTION: In functional uterine bleeding, how does testosterone act on the proliferative endometrium to cause a cessation in bleeding?

ANSWER: Testosterone is not the hormone of choice in the treatment of functional uterine bleeding. It is of value in patients with uterine fibroids or endometriosis. Testo-

sterone in moderate dosage does not cause any changes in the endometrium. With very large doses, however, atrophy of the endometrium results. If and when testosterone is effective in arrest of bleeding, it is felt that this is due to the myotonic effect of the hormone on the uterus rather than to its action on the endometrium.

QUESTION: What is the basis of the positive reaction to the pregnancy test in such conditions as dysgerminoma or mesonephroma?

ANSWER: A positive reaction to the pregnancy test may be obtained in some cases of dysgerminoma or mesonephroma but, as a rule, the reaction is negative. A mild positive reaction is not infrequently obtained in teratoid tumors (to which group these tumors belong) because, associated with embryonal tissue, there is increased prolan or gonadotropin-like hormone production.

QUESTION: In menopausal women who are undergoing pelvic surgery, is the routine removal of ovaries justified, on the basis of prophylaxis against carcinoma of the ovary? Are we sure that ovarian function ceases with the menses?

ANSWER: The routine removal of ovaries in menopausal women who are undergoing pelvic surgery is not justified on the basis of prophylaxis against carcinoma of the ovary. Complete atrophy of the ovaries does not occur with cessation of menses; some degree of ovarian activity continues for as many as a dozen years afterwards. Late in the postmenopausal period, with complete atrophy of the ovaries, removal of the ovaries is justifiable.

QUESTION: If androgens are given during the menopause, how much methyltestosterone can be given monthly without producing masculinization? Orally? Sublingually?

ANSWER: Androgens per se are not the hormones of choice in treatment of the menopause. However, androgens may be used when there are contraindications to estrogens and when undesirable side effects result from estrogen administration. Methyltestosterone may be given in doses of 10 mg. per day orally or sublingually for periods of two to three months, after which time a rest interval of one to two months is advised in order to avoid virilizing signs. Some patients are much more sensitive to androgens than others; one patient may take androgens more or less continuously for a period of from six to twelve months without virilizing signs, whereas another patient may show signs of virilization after two or three months of therapy. Therefore, the regulation of the dosage schedule should depend on individual responsiveness.

QUESTION: Is there anything new on idiopathic(?) vitiligo in females, relating to etiology and treatment on an endocrine basis?

ANSWER: Idiopathic vitiligo in females is still an enigma to endocrinologists. No endocrine therapy is presently available for treatment of this condition.

QUESTION: Would you use gonadotropins in cases of ovarian hypofunction secondary to pituitary insufficiency?

ANSWER: Theoretically, the use of gonadotropins in cases of ovarian hypofunction secondary to pituitary insufficiency should be ideal. In some instances the administration of pregnant mares' serum for from ten to fourteen days, followed by anterior pitiutary-like

hormones (chorionic gonadotropin) for another ten to fourteen days, results in ovarian stimulation with consequent menstruation. However, the menses which occur are, in almost every instance, anovulatory. Refractoriness soon develops to this form of treatment, and many patients do not respond at all to simple gonadotropin medication. Cyclic steroid therapy is to be preferred.

QUESTION: In cyclic therapy for ovarian deficiency (of either ovarian or pituitary origin), may the same dose be maintained, or must the daily dose be increased after from one to three months?

ANSWER: By "cyclic therapy for ovarian deficiency," it is assumed that the inquirer is speaking in terms of cyclic steroid therapy. As a rule, adequate cyclic therapy will result in improvement in the cosmetic appearance of the patient and also in withdrawal bleeding periods. In few patients will there be a need to increase the dosage levels because of failure to respond to previous levels. In most patients the dosage may be decreased after a time, because of increased sensitivity of the uterus to steroid hormones. In each patient, therapy must be gauged according to the individual response.

QUESTION: A patient has apparently normal sexual development and a normal pelvis; no menstruation; endometrial biopsy shows atrophic endometrium; no response to sex hormones. Classification? Therapy?

ANSWER: Patients with primary amenorrhea sometimes fail to respond to the first few courses of cyclic steroid treatment. In these cases the dosage should be greatly increased and therapy pursued with greater diligence. Withdrawal bleeding periods may be expected in most instances. Once menstruation has been induced, the dosage may be greatly reduced, and continued cyclic therapy will result in regular withdrawal bleeding periods. Occasionally, failure of target-organ responsiveness to intrinsic estrogens is encountered. In these instances there is usually evidence of good estrogen activity, such as excellent female habitus, good breast development and pubic hair growth, and a mature vaginal smear; nevertheless, there is uterine (endometrial) refractoriness. Such cases as these, as well as those of psychogenic amenorrhea, may prove to be hormone-resistant.

The Endocrine Society

ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-FIFTH MEETING

THURSDAY, FRIDAY AND SATURDAY, MAY 28, 29 and 30, 1953

Hotel Statler
New York, N. Y.

(Abstracts arranged according to numbers on the printed program)

THURSDAY, MAY 28, 1953

9:30 A.M.: GONADS, SEX HORMONES AND GONADOTROPINS

CHAIRMEN: WARREN O. NELSON AND C. L. BUXTON

1. Quantitative Aspects of Regression of Prostate and Uterus of the Rat Following Hormonal Deprivation.

Roy Hertz, M.D., Ph.D. and *William W. Tullner, B.S.* (by invitation).

From the National Cancer Institute, Bethesda, Md.

Estensive data permit the quantitative characterization of hormone-induced tissue growth in both the prostate and uterus of the rat. In contrast, little quantitative study has been made of the marked regression of the organs following hormonal deprivation resulting from gonadectomy, or from cessation of hormone administration in gonadectomized animals. We wish to report that this regressive process follows a highly characteristic and readily reproducible quantitative pattern. The curves present a very steep slope in the early phase of the involutionary process, and this is followed by a much less rapid secondary phase. This pattern is maintained without material change even in the presence of drastic metabolic alteration by such means as starvation, thyroidectomy, adrenalectomy, hypophysectomy, thyroxine or cortisone administration, and B-complex deficiency.

The data suggest that the character and speed of the regressive process is determined by factors within the involving organ rather than by changes in the internal environment. In view of the therapeutic importance of regression following hormonal deprivation by gonadectomy in cancer of the prostate and in cancer of the breast the hormonal mechanisms involved warrant detailed experimental analysis.

2. The Prolonged Anabolic Activity of Certain Testosterone Esters in Man.

R. Palmer Howard, M.D., Edward C. Reifenstein, Jr., M.D., Claiborne L. Courtright, M.D. (by invitation) and *Henry H. Turner, M.D.*

From the Section of Endocrinology and Metabolism, Oklahoma Medical Research Foundation; and the Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma.

It has been demonstrated that testosterone induces in man anabolism of protoplasm and bone, as indicated by retention of nitrogen, phosphorus and calcium during metabolic balance studies. In the present investigation, such studies employing standard procedures were carried out on 5 patients with osteoporosis, and the anabolism induced by testosterone propionate (T.P.) was compared in duration and amount with that induced by testosterone cyclopentyl propionate (T.C.P.) and testosterone phenyl acetate (T.P.A.). The effects of single large injections with equivalent testosterone content were evaluated. Each patient served as his own control; placebo injections were given at suitable intervals.

The anabolic manifestations were of relatively short duration after T.P. (under twelve days), whereas they were of considerably longer duration after T.C.P. (up to twenty-four days) and after T.P.A. (up to thirty-six days). The daily rate of anabolism was considerably greater with the longer-acting compounds. In the only study of repeated administration, the prolonged activity of T.C.P. was maintained by injections every twelve days.

Because of the increased duration and amount of effect, T.C.P. and T.P.A. appear to be more advantageous agents than T.P. for the management of conditions such as eunuchoidism, osteoporosis, or breast malignancy, in which prolonged testosterone administration is desired.

3. Elastic Fibers in the Tunica Propria of Normal and Pathologic Human Testes.

F. A. de la Balze, M.D., G. E. Bur, M.D. (by invitation) *Clorinda Scarpa Smith, M.D.* (by invitation) and *Juan Irazu, M.D.* (by invitation).

III Catedra de Clinica Medica de la Facultad de Medicina, y Instituto da Endocrinologia Regional, Buenos Aires, Argentina.

Biopsy material was obtained, placed in Bouin fixative and stained with Weigert. One hundred and sixteen human testes were examined.

Normal testes

Adults under 60 years of age (19 cases): Elastic fibers—thickness up to 1 micron, length variable, staining homogeneous, dark blue.

Adults over 60 years of age (11 cases). In a large percentage of tubules the elastic fibers were increased in number and thickness.

Newborn (4 cases): No elastic fibers.

Prepuberal boys (5 cases): No elastic fibers.

Puberty: The appearance of elastic fibers during puberty coincided with the maturation of the Leydig cells and the tubular content.

Pathologic testes

Prepuberal hypogonadotropic hypogonadism (7 cases): No elastic fibers.

Postpuberal hypogonadism produced by administration of estrogen (15 cases): Initially, short and thin fibers; later, intertwined fibers placed in the periphery of the sclerohyalinized wall.

Prepuberal hypergonadotropic hypogonadism (2 cases): No elastic fibers.

Puberal hypergonadotropic hypogonadism, Klinefelter syndrome (13 cases): Elastic fibers were observed in 6 cases only, these being the ones that had trophic Sertoli cells and spermatocytes.

Hypospermatogenesis and arrest of maturation (8 cases): Normal elastic fibers.

Syndrome of absence of germ cells (5 cases): Increase of elastic fibers.

Precocious true puberty (3 cases): Adult elastic fibers.
Mumps orchitis (8 cases) } Findings to be presented.
Brucellosis orchitis (16 cases) }
The various factors which may influence the development and maintenance of elastic fibers will be discussed.

4. The Effects on the Testes of Suppression of Adrenal Hyperplasia by Cortisone.

José Cara, M.D. (by invitation) and *Lawson Wilkins, M.D.*

From the Department of Pediatrics, Johns Hopkins University, School of Medicine, Baltimore, Md.

Testicular biopsies were made before and after cortisone therapy in 5 boys with macrogenitosomia praecox due to congenital adrenal hyperplasia. Before treatment the testes were small and immature and did not show Leydig cells. In some cases they remained immature after treatment; in others there was rapid increase in size, development of the tubules with spermatogenesis and appearance of Leydig cells. As in female pseudohermaphrodites, the gonadal maturation which occurs when the adrenals are suppressed with cortisone depends upon the patient's development level (as measured by the "bone age") rather than upon the chronologic age at the time of treatment.

5. The Histologic Response of the Gonads of Intact Immature Mice to Injections of Gonadotropins.

Helen Thomopoulou, M.D. (by invitation) and *Choh Hao Li, Ph.D.*

From the Laboratory of Fuller Albright, M.D., Massachusetts General Hospital, Boston, Mass. and the University of California, Berkeley, California.

Purified FSH and ICSH isolated from sheep pituitaries were administered subcutaneously to intact immature male and female mice. The effect of dose, duration of injection period, and combination of hormones was studied by the histologic appearance of the ovary, uterus, and testis. It was possible in the female to obtain a specific response to FSH, and to ICSH. The former consisted of an increase in the number and maturation of follicles; the latter of luteinization. There appeared to be synergistic activity when the two hormones were administered simultaneously. Corpora hemorrhagica were not observed in the dose range of FSH and ICSH studied. These gonadotropins were less effective in the male. ICSH had a greater effect upon spermatogenesis and Leydig-cell maturation but the response was qualitatively identical to that obtained with FSH. By controlling the dose and duration of injections it was possible to produce follicular growth or corpora hemorrhagica in the ovaries of mice treated with chorionic gonadotropin (APL) of human origin.

6. The Effects of Hog Pituitary Gonadotropin Therapy and Subsequent Antihormone Formation on Ovarian Function.

William O. Maddock, M.D., Ph.D., Ichiro Tokuyama, M.D. (by invitation), *Robert B. Leach, M.D.* (by invitation) and *William R. Roy, M.D.* (by invitation).

From the Departments of Medicine, and Obstetrics and Gynecology, Wayne University College of Medicine, Detroit, Michigan.

The administration of a hog pituitary extract, containing mainly FSH (12.5 or 25 rat units twice daily for one week to three months) resulted in a prompt increase in estrogen excretion to levels as high as fifty times pretreatment values in 4 patients hav-

ing functioning ovaries, but no increase in 2 men and 2 castrated women. Two patients received treatment for two and three months—a 7-year-old girl, having constitutional precocious puberty, and an 18-year-old patient, having severe idiopathic menorrhagia. Antihormones, which formed in both patients, were first detected during the second and third month of therapy. The patients' plasma, administered to immature female rats, prevented the action of human pituitary gonadotropin, human chorionic gonadotropin, and the administered hog FSH. Estrogen excretion fell during the second or third month of therapy to below pretreatment levels. In the precocious girl, menses ceased and estrogen excretion remained low for four months after stopping therapy; then estrogens increased and two regular menses ensued. Ten days after starting a second course of hog FSH, antihormones increased markedly and estrogen excretion fell to castrate levels. It is concluded that temporary cessation of ovarian function can be induced by eliciting antigonadotropin formation.

7. The Gonadotropin-Inhibiting Effect of an Augmentor Extracted from the Urine of Pregnant Mares.

Mia Parsonnet, M.D. (by invitation), *Herman Cohen, Ph.D.* (by invitation), *Leonard Hirsch, B.A.* (by invitation) and *Herbert S. Kupperman, M.D., Ph.D.*

From the Department of Therapeutics, New York University College of Medicine of the New York University-Bellevue Medical Center, New York, N. Y., and the Princeton Laboratories, Princeton, N. J.

The nonketonic, neutral material remaining in the toluene fraction obtained from the phenolic extract of pregnant mares' urine has been shown to augment the action of estrogen and possess a uterotropic effect despite the failure of the compound to induce vaginal cornification (Cohen & Bates: *Endocrinology* **50:** 5, 1952). The effect of such a preparation upon pituitary secretion is of interest in view of its failure to induce vaginal cornification in physiologic doses. The pituitary gonadotropin-inhibitory effect of this compound was studied in parabiotic rats. Each of a series of immature female rats, 31–33 days of age, was joined in parabiosis with a castrated littermate (male or female). The animals remained in surgical union for a period of ten days. The extent of the gonadotropic hypersecretion of the castrated parabiont's hypophysis was measured by the gonadal hypertrophy of the intact littermate female. The gonadotropin-inhibiting effect of the augmentor was determined by injecting 15–125 gamma of the material per day for ten days into the castrated partner. The administered material prevented in the left partner the gonadal hypertrophy noted in the uninjected control partner. This material possessed little or no estrogenic activity in the doses employed. A further study on the effect of the compound upon the gonadotropic secretion of the rat pituitary was demonstrated by the injection of the material (250 gamma per day) into rats recently mated. Termination of pregnancy of these animals was obtained by this dose. This compound proved to be a pituitary inhibitor in doses below those producing detectable estrogenic activity. The importance of a compound capable of inhibiting pituitary excretion in non-estrogenic doses in both experimental and clinical endocrinology will be discussed.

8. The Effect of Progesterone on the Lactic Dehydrogenase-DPNH Oxidase System of Ovariectomized Rat Uteri.

Arley T. Bever, Ph.D. (by invitation), *Joseph T. Velardo, Ph.D.* (by invitation) and *Frederick L. Hisaw, Ph.D.*

From the Biological Laboratories, Harvard University, Cambridge, Mass.

In a previous report (*Fed. Proc.*, 1953), estradiol-17β, estrone and estriol were demon-

strated to increase the lactic dehydrogenase-DPNH oxidase system activity of ovariectomized rat uteri as measured by neotetrazolium reduction. Estriol was least effective. When estriol and estradiol-17β were given together the enzyme system levels were reduced to that attained by estriol alone. In this report, the effect of progesterone on the system was investigated. Doses of progesterone between 0.1 and 1.0 mg., alone and in combination with 0.1 μgm. of estradiol-17β, were injected daily for three days into rats that were 100 days old, weighed 200 Gm. and had been ovariectomized one week. Control animals received no treatment. Daily doses of progesterone up to 0.5 mg. depressed the enzyme system to, or below, that of the nontreated group. One mg. daily produced an enzyme level between that of the nontreated group and a group receiving 0.1 μgm. of estradiol-17β per day. Estradiol-17β, when given with progesterone, did not raise the enzyme system level above the that of nontreated animals. Progesterone appears to act on this system by reducing the activity of the flavoprotein (DPNH oxidase) portion of the system. The lactic dehydrogenase component activity is increased by progesterone. This effect is opposed by estradiol-17β.

9. The Effect of Estrogen Pretreatment on Amino Acid Incorporation in Surviving Rat Uteri.

Gerald C. Mueller, M.D., Ph.D. (introduced by E. S. Gordon, M.D.).

From the McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.

At varied times after the administration of 10^{σ} of α-estradiol, rat uterine horns were removed and incubated in vitro with radioactive amino acids. During the first twelve hours the rate of glycine incorporation into uterine protein was accelerated in a linear manner, with significant effects apparent within two hours. Throughout the first six hours a maximal rate of response was elicited with 0.1γ of α-estradiol per 200-Gm. rat. Maximal activation (150 per cent stimulation) was observed at eighteen to twenty hours following a single dose of α-extradiol; thereafter the rate of incorporation declined toward the control level, due to a metabolic lowering of the estrogen level below that necessary for the continued activation. Similar effects of estrogen pretreatment were observed with the essential amino acids, tryptophane-β-C^{14} and lysine-2-C^{14}; however, alanine-2-C^{14} incorporation was not apparently altered when tested with a tracer dose of the latter. Pretreatment of the rats with lethal doses of Aminopterin did not prohibit the initial stimulating effect of estrogen treatment on glycine incorporation. The incorporation of orotic-6-C^{14}-acid into the uterine nucleic acids was not accentuated within the first six hours, at which time amino acid incorporation was accelerated. The relation of estrogen action to the stimulation of protein and nucleic acid synthesis will be discussed.

10. Some of the Hormones Required by the Mammary Gland in its Development and Function.

Wm. R. Lyons, M.D., Choh Hao Li, Ph.D., Roger D. Cole, B.S. (by invitation) and *Ruth E. Johnson, B.A.* (by invitation).

From the Department of Anatomy, School of Medicine, Department of Biochemistry, and Institute of Experimental Biology, University of California, Berkeley, California.

At the 1952 meeting we reported that estrone, progesterone, pituitary mammotropin

(M. H., prolactin) and somatotropin (S.T.H., growth hormone) injected for seven to ten days into hypophysectomized, oophorectomized rats induced lobulo-alveolar mammary growth similar to that of the latter half of pregnancy. This work has now progressed to the next phase, namely, a search for a combination of purified hormones necessary to initiate lactation in glands so-prepared.

One procedure now used is to hypophysectomize and oophorectomize 2½-month-old, Long-Evans rats and inject 10 I.U. of estrone, 4 I.U. of progesterone, 60 I.U. of M.H. and 0.1 mg. of S.T.H. daily subcutaneously for one week, in order to induce good lobular growth. Then during the second week the animals receive further daily treatment with M.H. and S.T.H. plus 10γ of thyroxin and 1 mg. of cortisone. Rats with intact adrenals may be given ACTH instead of cortisone.

The treatment during the second week causes a mitotic proliferation of the cells of the already-developed alveoli, a desquamation of some of the cells in a fluid considered to be colostrum, and finally, milk secretion.

The combination of hormones used in stimulating growth of the glands during the first week is effective in the absence of the adrenals, ovaries and pituitary, if the rats are given 1 per cent NaCl to drink.

TO BE PRESENTED IF TIME PERMITS

11. Inhibition by Estrogen of "Compensatory" Renal Hypertrophy in the Rat.

C. A. Schaffenburg, M.D. and *E. Perry McCullagh, M.D.*

From the Cleveland Clinic Foundation and the Frank E. Bunts Educational Institute, Cleveland, Ohio.

Since, on the one hand, the essential role of the anterior pituitary in the "compensatory" hypertrophy of the kidney has been well established and on the other hand, estrogens have been shown to be powerful pituitary inhibitors, it seemed of interest to study the effect of these hormones on the foregoing phenomenon. Two separate experiments were carried out using adult, unilaterally nephrectomized female rats, along with absolute, intact controls. Although estrogens in small, "physiological" doses (1.5 gamma/day, alpha-estradiol) had no effect on the "compensatory" hypertrophy of the sole remaining kidney, doses of 250 gamma per day caused a significant inhibition of this hypertrophy as compared to that occurring in pair-fed, unilaterally nephrectomized, untreated controls. Work is being continued to establish the significance of these findings in relation to the endocrine control of the kidney.

12. Human Urinary Gonadotropin in Pregnancy.

R. A. Lyon, M.D., M. E. Simpson, Ph.D., M.D. and *H. M. Evans, M.D.*

From the Institute of Experimental Biology, University of California, Berkeley, California.

Human gonadotropin titers (m.u.u.) increased notably by the tenth day following ovulation (basal body temperature method). Tested in immature hypophysectomized rats, such urines extracted by a revised method produced only interstitial repair and hypertrophy for the first sixteen days. After nineteen days, follicle stimulation was observed. The highest concentration (ovarian weights and histology) was present between post-ovular days 26 and 56, but a follicle-stimulating substance was detected as late as 238 days after ovulation.

13. The Influence of Physiologic Doses of the Sex Steroid Hormones on the Serum Lipids and Lipoproteins in Men and Women.

S. J. Glass, H. Engelberg (by invitation), *J. Gofman* (by invitation) and *H. B. Jones* (by invitation).

From the Cedars of Lebanon Hospital, Los Angeles and the Division of Medical Physics, University of California, Berkeley, California.

Since there are sex differences in atherosclerosis as well as in some aspects of human lipid metabolism, it was of interest to study the effect of the sex steroid hormones on the lipid constituents of the blood in men and women. To this end 31 subjects were given buccal tablets of estradiol, methyltestosterone and progesterone, singly and in combination, for several months in physiologic doses. Determinations were made of cholesterol, cholesterol esters, phospholipids, and of the lipoproteins (by ultracentrifugal analysis) before the onset of therapy, at monthly intervals thereafter and in some cases one month after the cessation of therapy. Deviations from control values were occasionally obtained after physiologic doses of these hormones but the changes were inconstant and not statistically significant. It was concluded that physiologic doses of the sex steroid hormones orally administered do not induce changes in the serum lipids and lipoproteins of adult human subjects.

2:00 P.M.: ADRENALS AND ACTH

CHAIRMEN: PETER H. FORSHAM AND LAURANCE W. KINSELL

14. Selective Arrest in the Development of One Extremity by Treatment with Hydrocortisone.

Hans Selye, M.D.

Institut de Médecine et de Chirurgie expérimentales, Montreal, Canada.

In rats (30 to 40 Gm.), on the first, tenth and twentieth days of the experiment, 2.5 mg. of hydrocortisone acetate microcrystals were injected into the right hind paw, while the left hind paw was similarly treated with microcrystals of DCA. By the thirtieth day, the left paw had increased in size in proportion to the body weight, while the right paw remained almost as small as it had been at the beginning. The stunting of growth, by hydrocortisone affected all parts of the right paw (bones, tendons, muscles, vessels, skin, nails) rather evenly, although the hormone deposit was limited to a restricted area. There was no detectable interference with the functional value of the hydrocortisone-treated paw (walking, grasping). In a second, similar experiment, the rats also received 0.5 mg. of somatotropic hormone (STH) subcutaneously. This increased somatic growth as a whole, but the hydrocortisone-treated paw was still subnormal.

It is concluded that suitable topical treatment with hydrocortisone can produce a kind of "post-natal malformation" of a limb, resulting in a proportionate, but selective, inhibition of its various constituents; this growth anomaly is not rectified by comparatively high doses of STH.

15. Studies on Androgen Production by the Human Adrenal Cortex.

Paul L. Munson, Ph.D., John C. Laidlaw, M.D. (by invitation), *Frederick C. Goetz, M.D.* (by invitation), *J. Hartwell Harrison, M.D.* (by invitation) and *George W. Thorn, M.D.*

From the Harvard School of Dental Medicine, the Department of Medicine and Surgery, Harvard Medical School, and the Medical and Surgical Services, Peter Bent Brigham Hospital, Boston, Mass.

Urinary excretions of 17-ketosteroids and androgens (measured by the chick-comb methods of Ratoff *et al.*) have been estimated in orchidectomized patients with metastatic prostatic carcinoma, prior to, and following complete bilateral adrenalectomy. Following adrenalectomy, with patients maintained on desoxycorticosterone acetate only, a definite although much reduced 17-ketosteroid and biologically active androgen excretion persists. Maintenance doses of cortisone (25–50 mg. daily) do not increase androgen excretion above this level. With larger doses of cortisone the increase in androgen excretion is proportional to the dose of hormone. The steroids tested may be listed tentatively in descending order of potency as precursors of androgens and 17-ketosteroids as follows: hydrocortisone, cortisone, corticosterone. Even with hydrocortisone in a dose of 300 mg. per day, however, androgen excretion is far below that of the average normal male, although 17-ketosteroid values are above normal. The androgen activities of specimens containing approximately equivalent levels of 17-ketosteroids attained prior to adrenalectomy (with ACTH) and following adrenalectomy (with hydrocortisone) are found to differ appreciably. These observations support the concept that androgens or androgen precursors other than hydrocortisone, cortisone and corticosterone are liberated by the intact adrenal.

16. Importance of the Liver in Transformations of Administered Adrenosteroidal Compounds.

Stefan S. Fajans, M.D. (by invitation), *Lawrence H. Louis, Sc.D.* (by invitation), *Holbrooke S. Seltzer, M.D.* (by invitation) and *Jerome W. Conn, M.D.*

From the Department of Endocrinology and Metabolism, University of Michigan Medical School, Ann Arbor, Michigan.

The adrenosteroidal compounds F, F acetate, E acetate, B, and S acetate have been administered to normal subjects at dosage levels of 200 and 400 mg. per day. During oral administration of each compound the urinary excretion of 17-ketosteroids was significantly greater than that observed during intramuscular administration of the same compound in the same amount. This interesting phenomenon was not observed with respect to excretion of formaldehydrogenic steroids. Oral administration of compounds or its acetate to patients with liver disease caused a very much smaller increase in excretion of 17-ketosteroids than that observed when the steroid was given to normal subjects. These results suggest 1) that the liver plays a major role in the transformation of steroids to 17-ketosteroids, 2) that the damaged liver is incapable of performing this function normally, 3) that when corticosteroids are administered orally, their passage through the liver in high concentration is responsible for their greater transformation to 17-ketosteroids, and 4) that certain unusual patterns of urinary steroids observed in a number of clinical conditions are the result of impaired hepatic transformation of endogenous steroids.

17. The Effect of Cortisone upon the Fatty Liver and Kidney Changes of Choline-Deficient Rats.

Elizabeth L. Hurd, B.A. (by invitation), *Florence K. Bass, B.S.* (by invitation), *Arthur C. DeGraff, M.D.* (by invitation), and *Herbert S. Kupperman, M.D., Ph.D.*

From the Department of Therapeutics, New York University College of Medicine of the New York University-Bellevue Medical Center, New York, N. Y.

The effect of cortisone and adrenal cortical extract in liver disease has been reported in various clinical studies. Since the method of action of the adrenal steroids in these cases is obscured, the effect of cortisone and adrenal cortical extracts was studied in rats with experimental liver disease. Fatty liver changes were induced through the use of a choline-deficient diet in male weanling rats weighing 34–45 grams. The treated animals received subcutaneous doses of cortisone and Lipo-adrenal cortex upon initiation of the diet. These animals, together with untreated diet control rats and animals on a normal diet, were killed after seven days. Liver fat was determined chemically and histologic sections of the liver and kidney of all animals were examined for evidence of fatty infiltration. Two mg. of cortisone per day prevented fatty liver changes and distortion of the kidney cytoarchitecture. Lipo-adrenal cortex and smaller doses of cortisone were less effective. The average percentage of liver fat for the animals on a normal diet was 5.1 (25 rats), as compared with 17.7 (32 rats) on a choline-deficient diet. Liver fat in the animals on 1.0 and 2.0 mg. of cortisone a day was 11.5 per cent (4 rats) and 6.5 per cent (23 rats), respectively, as compared with 14.8 per cent (15 rats) and 20.5 per cent (4 rats) for the animals on 0.5 cc. and 1.0 cc. of Lipo-adrenal cortex, respectively. Two mg. of cortisone a day, however, did not cause reversal of fatty liver changes, when such therapy was initiated after fatty liver changes had been previously induced by a choline deficient diet. The percentage of liver fat in rats on 2 mg. of cortisone per day, administered one week after the animals had been started on a choline-deficient diet, was 16.7 per cent (19 rats) as compared to the two-week diet control value of 21.5 per cent (19 rats). A discussion of the factors involved in the effect of cortisone upon choline-deficient diet and the therapeutic application will be presented.

18. The Simultaneous Measurement of Exchangeable Sodium and Potassium Spaces in Man.

Walter L. Arons, M.D. (by invitation), *Robert J. Vanderlinde, M.D.* (by invitation), and *Arthur K. Solomon, M.D.* (by invitation) (introduced by George W. Thorn, M.D.).

From the Department of Medicine and the Biophysical Laboratory, Harvard Medical School, and the Medical Service, Peter Bent Brigham Hospital, Boston, Mass.

A method for the simultaneous measurement of the exchangeable sodium and potassium spaces in man has been devised, employing an ion-exchange resin for separation of these cations. Twenty-four hours after the intravenous administration of tracer amounts of radioactive sodium and potassium a serum sample is drawn. The sodium and potassium of the serum are then separated by differential chromatographic elution with 0.3 normal hydrochloric acid, on an ion exchange column (Dowex 50, 12 per cent cross linked, minus 325 mesh, 6 cm. in height). The specific activities of the elution fractions are determined and, after correcting for urinary losses, the exchangeable sodium and potassium spaces are calculated by use of the standard isotope dilution formula. Successive combined space determinations in normal subjects prove the method to be highly reproducible.

This method is being utilized to determine the effect of the administration of ACTH and adrenocortical steroids on the exchangeable sodium and potassium spaces. In pa-

tients in metabolic balance, exchangeable sodium and potassium spaces are measured before and at varying intervals during the administration of large doses of the hormone being studied. An analysis of the space measurements in relation to the external balance of sodium and potassium will be presented.

19. Water and Salt Metabolism During Acute Adrenal Insufficiency in Man.

Olof H. Pearson, M.D., Mortimer L. Mendelsohn, M.D. (by invitation) and *Charles D. West, M.D.*

From the Sloan-Kettering Institute, New York, N. Y.

Totally adrenalectomized patients are adequately maintained with cortisone as the only hormone replacement. Cortisone was withdrawn for three to seven days in 7 adrenalectomized patients while on a metabolic balance regimen. In 2 patients, body fluid compartments were measured by dilution techniques before and during cortisone withdrawal. Clinical collapse developed promptly in all patients when cortisone was withdrawn, and rapid recovery occurred when the hormone was restored.

During cortisone withdrawal, urinary sodium loss was conspicuously absent in 4 patients. Significant hyponatremia developed in 4 patients, which could not be explained by loss of body sodium. Four patients gained weight during cortisone withdrawal. Potassium retention was not observed in 5 patients on whom these measurements were made. Serum potassium concentration remained normal in 6 patients. There was an apparent expansion of the inulin space of 15 to 21 per cent, without significant change in the total body water or total exchangeable body sodium during cortisone withdrawal in the 2 patients studied. When cortisone was readministered, 6 patients promptly lost 1 to 3 kilograms of body weight due to water diuresis.

These results are at variance with experimental data in animals and the classic concept of the salt-losing Addisonian patient. They provide a new physiologic concept for the treatment of acute adrenal insufficiency in man.

20. The Anti-Anabolic Effect of Cortisone on the Anabolic Property of Intravenously Administered Serum Albumin.

Philip H. Henneman, M.D., A. P. Forbes, M.D., Eleanor F. Dempsey, M.A. (by invitation) and *Fuller Albright, M.D.*

(Withdrawn from program by the authors.)

21. Physiologic Effects of Corticosterone in Man.

Charles D. West, M.D., Olof H. Pearson, M.D. and *Attallah Kappas, M.D.* (by invitation).

From the Sloan-Kettering Institute, New York, N. Y.

A dose of 100 to 200 milligrams of oral corticosterone daily maintained a totally adrenalectomized patient satisfactorily for nine days without other hormonal replacement. Withdrawal of corticosterone resulted in prompt clinical collapse, and recovery from acute adrenal insufficiency was effected with 100 milligrams of oral corticosterone daily alone.

A dose of 100 to 800 milligrams of corticosterone was given daily for twenty-four days by mouth to another patient, while in metabolic balance. Corticosterone produced

a profound retention of sodium, chloride, and water, whereas no significant alteration in protein and carbohydrate metabolism occurred. Formaldehydogenic steroid excretion increased markedly on corticosterone. Urinary excretion of neutral C^{19} 11-oxy- and 11-desoxy-α-ketosteroids was normal during the control. On corticosterone, the excretion of these steroids decreased to such an extent that their detection by infra-red spectrometry was impossible.

A dose of 300 to 600 milligrams of corticosterone was given daily for six days intravenously to another patient. Corticosterone produced a slight increase in nitrogen, phosphorus, and potassium excretion, marked retention of sodium, chloride, and water, but no measurable effect on glucose metabolism.

These observations demonstrate that corticosterone has life-maintaining properties in man, profound salt-retaining effects, a slight protein catabolic effect, and no demonstable effect on carbohydrate metabolism. These effects will be compared with those of cortisone, hydrocortisone, and desoxycorticosterone in man.

22. Functional Evidence in Man for the Existence of an Adrenal Growth Factor Distinct from ACTH.

G. W. Liddle, M.D. (by invitation), *A. P. Rinfret, Ph.D.* (by invitation), *J. Richard, B.S.* (by invitation) and *P. H. Forsham, M.D.*

From the Metabolic Unit, the Department of Medicine, University of California School of Medicine, San Francisco, California, and the Department of Chemistry, Stanford University, Palo Alto, California.

Porcine and equine pituitary fractions (AGF) have been prepared, which maintain adrenal weight in hypophysectomized rats without leading to adrenal ascorbic acid depletion in the Sayers assay for ACTH. In contrast to conventional ACTH, the intravenous administration of AGF to human subjects results in practically no immediate increase in adrenal steroid secretion as estimated by urinary 17-hydroxycorticoids and 17-ketosteroid excretion, or changes in circulating eosinophils. In a series of 6 human subjects, adrenal responsiveness was tested on intermittent days by determining the increases in urinary 17-hydroxycorticoids induced by standard doses of ACTH given intravenously over eight-hour periods. These responses were found to be reproducible from test to test for any given subject. Each subject was then treated for five successive days with AGF. Repetition of the standard ACTH tests at this time resulted in urinary steroid responses which were two to three times as great as those obtained prior to treatment with AGF. This increased responsiveness gradually waned over the ensuing one to two weeks. Purified ACTH and somatotropic hormone preparation failed to produce a similar increase in adrenal responsiveness when administered on an identical schedule.

23. Relationship of Renal Excretion of Biologically Active Adrenal Corticoids to Variations in Glomerular Filtration Rate.

Leon J. Marks, M.D. and *Alexander Leaf, M.D.* (introduced by N. B. Talbot, M.D.).

From the Massachusetts General Hospital, Boston, Mass.

Clinical observation with the use of Speirs' urinary bioassay revealed that in a variety of stressful situations no urinary adrenal corticoids were demonstrable. These cases showed marked reductions in endogenous creatinine clearances. To test the possible role

of altered renal hemodynamics in the excretion of adrenal corticoids, studies were performed on dogs in whom controlled variations in renal blood flow and filtration rate could be produced unilaterally by compression of one renal artery. The opposite kidney served as control. Results revealed that with reduction of glomerular filtration rate there occurred a linear decrease in excretion of biologically active adrenal corticoids. Tubular reabsorption of hormone was suggested by the proportionately greater reduction in corticoid excretion than in glomerular filtration rate. It is suggested that this mechanism would serve to conserve hormone to the organism in some instances of stress. The importance of this relationship to the interpretation of values of urinary corticosteroid excretion is emphasized.

24. Influence of Stress on the Action of Cortisone.

K. E. Paschkis, M.D., A. Cantarow, M.D., A. M. DeAndino, Jr., M.D., D. Epstein, M.D. (by invitation), *W. Abelove, M.D.* (by invitation), and *E. Mazur, M.D.* (by invitation).

From the Division of Endocrine and Cancer Research, Jefferson Medical College, Philadelphia, Pa.

Until recently, the actions of cortical hormones were visualized almost exclusively in terms of quantity of hormone secreted or administered. Recently Ingle has introduced the concept that the response to cortical hormone depends in some way upon the condition of the organism, perhaps on "need for hormone."

Experiments to be reported are part of a study of the influence of various factors on different parameters of cortical hormone action. Adrenalectomized mice injected with a standard dose of cortisone were subjected to a variety of stresses. The thymolytic effect of cortisone under these conditions was compared with the thymolytic cortisone effect in non-stressed, adrenalectomized controls. Starvation, injection of formalin and of histamine diminished the thymolytic effect of cortisone. A slight reduction of the thymolytic effect of cortisone was observed with epinephrine. Similar results were obtained in experiments on the cortisone-induced inhibition of cutaneous spread.

TO BE PRESENTED IF TIME PERMITS

25. Studies on the Adrenal Cortical Response to Stress in Man.

Dalton Jenkins, M.D. (by invitation), *John C. Laidlaw, M.D.* (by invitation), *William J. Reddy, A.B.* (by invitation), *Lewis C. Mills, M.D.* (by invitation) and *George W. Thorn, M.D.*

From the Department of Medicine, Harvard Medical School and the Medical Service, Peter Bent Brigham Hospital, Boston, Mass.

Changes in urinary 17-hydroxycorticoid excretion and circulating eosinophils produced by graded doses of intravenous ACTH have been employed to establish dose-response and time-response curves in normal subjects. Continuous infusions of ACTH at dosage rates as low as 0.25 U.S.P. units per hour regularly produce both eosinopenia and a rise in urinary 17-hydroxycorticoid output. Below this level, neither index is consistently altered. Although eosinopenia following ACTH reflects adrenal cortical activation, a significant fall in eosinophils during a variety of states of "stress" often occurs without a concomitant rise in adrenal steroid excretion. Epinephrine administration also produces eosinopenia in normal subjects in the absence of increased urinary 17-

hydroxycorticoid output and commonly induces an eosinophil fall in patients who have undergone complete bilateral adrenalectomy. This constitutes additional evidence for mechanisms of eosinopenia independent of increased adrenal cortical hormone secretion. The urinary excretion of 17-hydroxycorticoids has been investigated in adrenalectomized patients and in patients with Addison's disease during constant intravenous infusions of adrenal steroids, during control periods and again during major stress. Minimal changes in the excretion pattern under these circumstances have been observed. The quantitative aspects of adrenal secretion and corticosterone "utilization" during stress will be discussed.

26. The Influence of Dietary Factors on the Hyaluronidase Spreading Reaction in Intact and Adrenalectomized Rats.

Edith M. Beck, M.D. (by invitation) and *Elaine P. Ralli, M.D.*

From New York University College of Medicine, New York, N. Y.

The hyaluronidase spreading reaction, using India ink as the indicator dye, was tested in intact and adrenalectomized rats fed either a dog chow diet, an experimental diet adequate in all nutritional factors for the rat, a diet containing large amounts of pantothenate, a diet deficient in pantothenate, or a diet containing large amounts of vitamin B_{12}. The spreading reaction was also tested in rats on the dog chow diet subjected to sham operations.

In intact rats the spreading reaction was significantly inhibited in the rats receiving large amounts of vitamin B_{12}. The other dietary situations had no effect on the spread of hyaluronidase.

Adrenalectomy was associated with a decreased area of spread in all diet groups, although it did not significantly further decrease the area of spread in the rats on the high vitamin B_{12} diet.

Up to five days after the sham operation, the spreading reaction was decreased to the same extent as in five-day adrenalectomized rats. However, sixteen days postoperatively, the area of spread was more depressed in the adrenalectomized rats than in those subjected to sham operation.

The data suggest that the spreading reaction of hyaluronidase is influenced by factors other than the adrenal.

27. The Influence of Starvation and the Adrenal Upon Iron Storage in the Rat.

Diana F. Silber, M.S. (by invitation) and *Albert S. Gordon, Ph.D.*

From the Department of Biology, Washington Square College of Arts and Sciences, New York University, New York, N. Y.

The effects of acute starvation, adrenalectomy and the administration of adrenal cortical extracts were studied upon the storage of iron, visualized by the Prussian Blue method, in the fixed macrophages of the spleen, liver, lymph nodes and thymus of the rat. Starvation results in a significant increase in the numbers of hemosiderin-containing macrophages and in the amount of hemosiderin within each cell. This effect is most pronounced in the spleen. It occurs to a more variable extent in the lymph nodes, and to the least extent in the liver and thymus. Adrenalectomy produces an iron-storage pattern in the spleen and lymph nodes, similar to that seen in the starved animal. In the adrenalectomized animal subjected to starvation, the quantity of hemosiderin deposited in the spleen is greater than that seen in either state alone. Administration of adrenal cortical

extracts to the starved or fed animals produces no striking additional alterations in iron deposition in the reticulo-endothelial organs. The enhanced iron deposition seen within the reticulo-endothelial system of the starved and adrenalectomized rat may be due to increased blood destruction in these two states.

FRIDAY, MAY 29, 1953

9:00 A.M.: STEROIDS AND BIOASSAY METHODS

CHAIRMEN: LEO T. SAMUELS AND IRA T. NATHANSON

28. The Estrogenic Activity of Urinary Preparations: Studies of Ultrafiltration, Alcohol Precipitation, and Kaolin Absorption.

H. M. Lloyd, B.M. (Oxon.), *A. J. Popert, M.B.* (London) and *E. F. Scowen, M.D.* (London) (introduced by Fuller Albright, M.D.).

From St. Bartholomew's Hospital, London, England.

Differences in "gonadotropic activity" of ultrafiltration extracts and alcohol precipitates of urine have frequently been found and are especially obvious during treatment with stilbestrol. Ultrafiltration extracts may then be highly active, but alcohol precipitates inactive. The method of assay depends on the increase in weight of the uterus of the intact immature female mouse and does not differentiate between gonadotropin and estrogen.

Results are presented to show that ultrafiltration extracts of urine from patients receiving stilbestrol are frequently estrogenic, as demonstrated by uterine weight in oophorectomized immature female mice, whereas alcohol precipitates are not estrogenic.

Of 5 men receiving large doses of stilbestrol for prostatic carcinoma, 3 gave ultrafiltration extracts which were estrogenic. Studies of a female patient suffering from hypopituitarism revealed estrogenic activity in the ultrafiltration extracts, but only at the beginning of treatment with stilbestrol. An obese postmenopausal woman was treated by starvation for seven days, following which her gonadotropin excretion fell to low values. A fourteen-day course of stilbestrol resulted in no estrogenic activity in the ultrafiltration extracts until the day after cessation of treatment.

Kaolin adsorption has been compared in some of these studies: in no instance have kaolin extracts been found to be estrogenic.

29. Bioassay of Adrenal Corticoids by Means of a Thymus Weight Response in Weanling Rats.

N. R. Stephenson, Ph.D. (introduced by L. I. Pugsley, Ph.D.).

From the Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada.

Involution of the thymus gland of intact weanling fats has been employed as the criterion of the response to hydrocortisone-like steroids. A linear relationship has been found to exist between the logarithm of the dose and the weight of the thymus gland over the dose range of 100 to 400 micrograms of hydrocortisone. The hormone preparation dissolved in oil, is administered by subcutaneous injection twice daily for three days. The thymus glands are removed and weighed on the morning of the fourth day. The slope of the log. dose-response line obtained with the intact animals does not appear to differ significantly from that observed with adrenalectomized rats of the same age and weight. The biologic activity of the adrenal cortex extracts as determined by thymic

involution is similar to that measured by the glycogen deposition type of assay. However, the variation within dosage groups is smaller in the thymus weight assay than it is in the glycogen deposition assay, whereas the ability of the test animals to discriminate between the doses appears to be approximately equal in the two procedures. Accordingly, the index of precision of the thymus involution test compares favorably with that obtained for the liver glycogen method.

30. A Clinically Significant ACTH Assay Based Upon Urinary 17-Hydroxycorticoid Excretion in Guinea Pigs.

G. W. Liddle, M.D. (by invitation), *D. Island, B.S.* (by invitation), *M. Dimitman, B.S.* (by invitation) and *P. H. Forsham, M.D.*

From the Metabolic Unit, University of California School of Medicine, San Francisco, California.

Evidence is presented showing that at least two conditions make it impossible to predict, on the basis of the adrenal-ascorbic-acid-depletion assay alone, the effectiveness of a given ACTH preparation when administered extravascularly to man. First: certain preparations of ACTH which exhibit no ascorbic-acid-depleting activity nevertheless prove to be steroidogenic agents in man. Second: extravascular administration of ACTH to human subjects results in variable inactivation of the ACTH, ranging from complete inactivation to complete preservation of activity. An assay method has been devised using the percentage-rise in urinary 17-hydroxycorticoids of guinea pigs during ACTH administration, which was found to be proportional to the logarithm of the dose of ACTH, in the dose range yielding steroid increases of from 50 to 200 per cent. This relationship follows a uniform slope (index of precision better than 0.1). By this assay an over-all evaluation of the effectiveness of extravascular ACTH is obtained which includes any functional-growth activity as well as the steroid-discharging activity of the ACTH and which, at the same time, corrects for the susceptibility of the preparation to local inactivation. This method makes it possible to predict accurately the potency of various ACTH preparations when used extravascularly in man.

31. Fractionation by Countercurrent Distribution of Fluorometrically Measured Urinary Estrogens.

Claude J. Migeon, M.D. (introduced by Lawson Wilkins, M.D.).

From the Department of Pediatrics, Johns Hopkins Hospital, Baltimore, Md.

A single countercurrent distribution of the phenolic fraction of urine often failed to reveal distribution of fluorogenic activity characteristic of the three natural estrogens, due to the presence of large amounts of nonspecific fluorogens. By a series of fractionations using several systems of solvent, however, it was possible to measure the amounts of estrone, estradiol and estriol in the urine of 2 normal males, 2 normal females, 1 pregnant female, 2 patients with adrenocortical tumor of the virilizing type and 4 patients with congenital adrenal hyperplasia before and during cortisone therapy.

There was a rather constant ratio between the three estrogens in normal individuals and in patients with congenital adrenal hyperplasia before and during adrenal suppression. An increase was suggested in the percentage of estriol in pregnancy and adrenocortical tumor.

Except in pregnancy only 19–41 per cent of the total fluorescence was accounted for by the amounts of the three known estrogens found. From the nonspecific fluorescent material, it was possible to separate two substances. One, designated as E^4, represented

7 to 15 per cent of the total fluorescence; another, designated E^5, represented 2.5 to 7.3 per cent. Their chemical composition or biologic activity has not been determined.

32. Metabolism of Isotopic Estrone in Human Pregnancy.

W. H. Pearlman, Ph.D., M. R. J. Pearlman, A.B. (by invitation) and *A. E. Rakoff, M.D.*

From Jefferson Medical College, Philadelphia, Pa.

6,7-d^2Estrone acetate (6.6 atom % excess deuterium) was administered to 2 women in advanced pregnancy. The deuterium content of the major urinary estrogens was determined with the aid of a mass-ratio spectrometer; the quantities of these estrogens were estimated by bioassay. The recovery of the administered isotopic estrone acetate in the form of urinary estriol was calculated to be 3.5 and 4.0 per cent (two experiments) and the total recovery of urinary estrogens was 6 to 7 per cent. Substantially the same figures were obtained in similar experiments which had been performed on men with normal estrone acetate. The percentage conversion (as distinct from recovery) of estrone to estriol in pregnancy was 3.4 to 3.6 per cent, whereas that of estrone to estradiol was 21 to 28 per cent. These results do not support the view of Smith and Smith (*Physiol. Rev.* **28**: 1, 1948), that progesterone decreases the rate of estrogen inactivation by facilitating the in vivo conversion of estrone to estriol. The physiologic significance of these findings will be discussed.

33. A New Method for Rapid Chromatographic Identification of Cortical Steroids; Application to Biologic Fluids.

Maurice Pechet, M.D. (introduced by Fuller Albright, M.D.).

From the Massachusetts General Hospital, Boston, Mass.

A new method for the rapid chromatographic analysis of ketol steroids is presented and its application to the determination of ketol steroids in urine is described. The method employs no pre-impregnation of the paper, since the developing solvent is monophasic with water as the stationary phase. A complete run requires only two hours. The results of application of this method to known keto steroids and individual cases will be discussed. Excellent separation of adrenal cortical hormones was obtained. A urine specimen from a 9½-year-old male child with acute leukemia treated with ACTH was extracted and chromatographed; it showed two spots identified as compound F and compound E. Serial dilutions and subsequent chromatography of aliquots of this urinary fraction permitted a semiquantitative estimation. In this manner 210 micrograms of compound F and 105 micrograms of compound E were found to be excreted in twenty-four hours. The results of other cases will be discussed. The merit of this method lies in its simplicity, rapidity and the ease with which individual ketol steroids can be separated, detected and estimated in semiquantitative fashion.

34. The Detection of Pregnandiol and Pregnantriol in the Urine of Patients with Adrenocortical Hyperplasia: Suppression with Cortisone.

Alfred M. Bongiovanni, M.D. (by invitation) (introduced by Lawson Wilkins, M.D.)

From the Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Md.

The presence of 3-α-17-α-20-pregnantriol and increased quantities of 3-α-20-pregnandiol in the urine of patients with the adrenogenital syndrome due to adrenal hyper-

plasia appears to be characteristic of this syndrome. A relatively simple screening procedure for the detection of pregnantriol has been devised, modifying the method of Sommerville and Marrian for pregnandiol. The determination of pregnandiol by this method may be influenced by contamination with pregnantriol. The administration of cortisone suppresses the urinary level of pregnandiol as well as of the 17-ketosteroids, and causes the disappearance of pregnantriol. There is a rapid reappearance of pregnantriol and a marked increase in the urinary level of pregnandiol with the administration of ACTH to patients previously receiving suppressive doses of cortisone. ACTH did not have this effect in 2 children with normal adrenocortical function. Since 17-hydroxyprogesterone may represent a precursor of pregnantriol, it was administered to 2 normal adult males. There was no evidence of the excretion of pregnantriol after three preliminary trials. The presence of pregnantriol, detected by the simple screening procedure to be described, was confirmed by the chromatographic isolation of this compound on alumina. The possible implications of these findings in relation to the disordered pattern of steroid synthesis in adrenocortical hyperplasia will be discussed.

35. Effect of ACTH and Various Substrates on *in vitro* Adrenal Cortical Steroid Synthesis.

Frederick G. Hofmann, Ph.D. (by invitation) and *Clarke Davison, M.S.* (by invitation) (introduced by Roy O. Greep, Ph.D.).

From the Biological Laboratory, Harvard School of Dental Medicine and Department of Biological Chemistry, Harvard Medical School, Boston, Mass.

Quartered adrenal glands from young adult female rats of the Sprague-Dawley strain were incubated in an artificial medium at 37° C. for two and one half hours. Ethyl acetate extracts of incubated and unincubated material were chromatographed on silica gel columns and an appropriate fraction analyzed for formaldehydogenic steroids. An average net steroid output under these control conditions was thus determined.

The addition of sodium acetate, glycerol, or sodium acetoacetate, in final concentrations of 10^{-3} and 10^{-2}M, significantly increased steroid production. Acetoacetate provided the greatest stimulus to steroid synthesis and its effect was far greater than that observed with the other two substrates.

ACTH (Astwood preparation), in a dose of 1 unit per 100 mg. of adrenal tissue, also significantly increased steroid production.

However, when both ACTH and any one of the aforementioned substrates were supplied to the glands simultaneously, steroid output was essentially that of the control incubation, and no stimulation was observed.

Steroid production ceased entirely when a sulfhydryl inhibitor, *n*-ethyl maleimide, in a final concentration of 10^{-2}M was added to the incubation mixture. This inhibition was not overcome by the addition of ACTH or by any of the substrates mentioned above.

36. Further Studies on Cortisone Metabolism in Perfused Rat Liver.

O. Hechter, Ph.D., E. Caspi, M.Ph. (by invitation) and *H. Levy, Ph.D.* (by invitation).

From the Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

In previous work, we have shown that isolated perfused rat livers rapidly transform cortisone and related corticoids like hydrocortisone and DOC to a variety of transformation products (Hechter, *et al., J. Clin. Endocrinol. & Metab.* **12**: 935, 1952). The present

work describes the isolation and characterization of some of the products derived from cortisone perfusion through rat liver. These include both 3α and 3β *allo* tetrahydrocortisone, 3β *allo* tetrahydro-17 hydroxycorticosterone, and (in trace amounts) Δ^4 pregnene 17α, 20,21 triol-11 one, adrenosterone and androstane 3α ol-11,17 dione.

Evaluation of the rates of hepatic utilization of corticoids, taken in conjunction with the nature of the transformation products, indicates that corticoids are powerful hydrogen acceptors under the conditions of our *in vitro* experiments.

Finally, in line with our previous suggestion that some cortisone transformation products retain biologic activity, studies were undertaken to test the activity of cortisone metabolites. These studies conclusively demonstrate the presence of corticoid activities in our products, which are not due to either cortisone or hydrocortisone contaminations.

37. Fate and Excretion of Exogenously Administered Cortisone.

Don H. Nelson, M.D. (by invitation) and *George W. Calver, M.D.*

From the U. S. Naval Medical School and the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.

Blood levels of 17-hydroxycorticosteroids have been determined simultaneously with urinary excretion of 17-hydroxycorticosteroids and formaldehydogenic steroids. Approximately 50 mg. of a 100-mg. dose of cortisone given orally to a normal subject is recovered in the urine as glucuronide conjugated 17,21-dihydroxy-20-ketones within twelve hours of the time the cortisone is given. Almost all of this recoverable fraction is excreted in the urine during the first six to eight hours, which is the time required for the initially very high blood level of 17-hydroxycorticosteroids to return to normal. Although significantly more formaldehydogenic steroids than 17,21-dihydroxy-20-ketones are found to be present in normal urine there is slight difference between quantities of these compounds secreted following a large dose of cortisone. Thus the ketol side chain and hydroxyl group at position 17 of the molecule have not been altered but the steroid has been largely conjugated as the glucuronide before excretion.

38. Disappearance of Progesterone from the Blood Following Intramuscular and Intravenous Injections of the Hormone.

R. L. Shoger (by invitation) and *M. X. Zarrow.*

From the Department of Biological Science, Purdue University, Lafayette, Indiana.

Progesterone concentrations in the blood of the rabbit were determined following intravenous or intramuscular injections of the hormone. In all instances, the Hooker-Forbes bioassay technique was used and aliquots of the serum assayed directly for free progesterone, or hydrolyzed for the determination of the protein-bound progesterone.

The intramuscular injection of 10 mg. of progesterone produced a peak of 1 μg. per ml. of serum within a half hour and a complete loss by eight hours. Following the intramuscular injection of 40 mg. of progesterone, a concentration of 2 to 4 μg. per ml. of serum was obtained, with a drop to zero by twelve hours. A microcrystalline suspension or a propylene glycol-albumin solution of progesterone was injected intravenously at a level necessary to produce a concentration of 10 μg. of progesterone per ml. of plasma. Approximately 80 per cent of the hormone was lost from the blood within five minutes and by twenty minutes no detectable progesterone could be found in the circulation. Studies

on the nephrectomized rabbit indicated a lag in the rate of disappearance of progesterone from blood following intravenous injections. In the absence of the kidney, only 60 per cent of the injected hormone was lost within five minutes and approximately twenty-four hours was needed for its complete disappearance. The rapid loss of progesterone from the blood could not be ascribed to protein binding.

TO BE PRESENTED IF TIME PERMITS

39. A Standard ACTH-Adrenal Steroid Response Test. Evaluation in Children.

Vincent C. Kelley, M.D., Ph.D. and *Robert S. Ely, M.D.* (introduced by Leo T. Samuels, Ph.D.).

From the Department of Pediatrics, University of Utah College of Medicine, Salt Lake City, Utah.

Clinical evaluation of the functional status of the adrenal cortex has been dependent upon indirect technics, such as hematologic responses and urinary excretion of steroids. The introduction of the ACTH-eosinophil response test has provided a simple indirect method of evaluating adrenal cortical responsiveness clinically. The method presented here provides a more direct approach to this problem, in that circulating concentrations of plasma 17-hydroxycorticosteroids have been employed as the criterion of adrenal responsiveness to ACTH stimulation. The data to be presented illustrate the responses observed in normal children, and in children with a variety of acute or chronic illnesses. The steroid responses in these groups are compared to their eosinophil responses. The data indicate that in most disease states there is no impairment in the ability of the adrenal cortex to respond to the ACTH stimulation used, although occasional individuals in this category fail to respond adequately. However, many children with epilepsy have evidence of an impaired adrenal cortical response. Individuals with clinical evidence of adrenal insufficiency consistently fail to respond to ACTH stimulation. Interestingly, children with rheumatic fever receiving continuous hormone therapy over a period of several weeks do not lose their ability to respond to ACTH stimulation. The method appears capable of detecting severe impairment in adrenal responsiveness. Without further refinement it cannot detect relative impairment of adrenal cortical function. In certain instances it appears to be more reliable than the ACTH-eosinophil response test.

40. Peritoneal Fluid as a Site for the Study of Endocrine Effects on Blood Cells.

Jacques Padawer, M.S. (by invitation) and *Albert S. Gordon, Ph.D.*

From the Department of Biology, Washington Square College of Arts and Science, New York University, New York, N. Y.

Rat peritoneal fluid contains large numbers of eosinophil leukocytes, macrophages and mast cells. An account is presented of the effects of pregnancy and some endocrine deficiency states on the volume and absolute cellular numbers of peritoneal fluid. The extent of eosinophilic leukocyte breakdown, phagocytosis by free macrophages, and the morphology of the mast cell have also been examined in these conditions. Pregnancy results in an increase in the absolute cell numbers and the volume of peritoneal fluid. Marked eosinophil breakdown and increased phagocytic efficiency of the macrophages are evident in late pregnancy; mast cells appear normal. Salt-maintained adrenalectomized rats (two weeks postoperatively) display increased peritoneal fluid volumes, smaller-sized mast cells and no significant changes in absolute cell numbers in peritoneal fluid. Hypophysectomy induces an increase in the size and distinct alterations in the shapes of

the mast cells. Eosinophilic leukocyte destruction and phagocytosis of degenerating eosinophils occur in both the hypophysectomized and adrenalectomized animal. In adrenalectomized rats subjected to lowered barometric pressures, the process of eosinophil destruction is accompanied by a pronounced rise in peripheral blood eosinophils.

41. Human Corticosterone Metabolism. III. The Urinary Pettenkofer Chromogen Increased by Corticotropin or Corticosterone is not Dehydroepiandrosterone.

William Q. Wolfson, M.D., Mioko Chambliss, B.S. (by invitation) and *William D. Robinson, M.D.* (by invitation).

From The Rackham Arthritis Research Unit, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan.

Urinary neutral Pettenkofer chromogens (PC) appear to be exclusively adrenocortical metabolites. Certain adrenal tumor patients show a disproportionately increased excretion of dehydroepiandrosterone (DHA) and a comparably increased excretion of PC. In the presence of adrenal tumor, the correlation between PC and DHA excretion appears excellent. Unfortunately, increases in Pettenkofer or Allen chromogen occurring regularly after corticotropin or corticosterone have also been interpreted as representing increased DHA excretion, even though no isolation data supported this view. Jailer, who separated high-Pettenkofer extracts from corticosterone-treated patients on alumina columns, was first to find that such extracts did not contain increased DHA. Similar results have now been obtained by paper chromatography. The high Pettenkofer extracts of urines from corticotropin and corticosterone-treated patients do not contain increased DHA. They do contain highly polar material which gives a three-spot chromatogram with the Pettenkofer reagent. The chromogens after corticosterone and corticotropin appear identical. Previous studies (*J. Clin. Endocrinol. & Metab.* **12**: 970, 1952) have shown corticosterone to be the only steroid capable of increasing PC excretion when given at physiologic levels. The present data suggest again that the PC increase after corticotropin is secondary to increased corticosterone secretion. Our findings are most consistent with the view that neither ACTH nor corticosterone causes a detectable increase in DHA excretion. The increased Pettenkofer chromogens excreted during corticotropin or cortiscosterone administration probably are closely related chromogenic derivatives of corticosterone.

2:30 P.M.: THYROID

CHAIRMEN: E. B. ASTWOOD AND ROBERT H. WILLIAMS

42. The Effects of Thyroxine and Triiodothyronine on Oxygen Utilization by Tissues *in vitro*.

John G. Wiswell, M.D. and *Samuel P. Asper, Jr., M.D.*

From the Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Md.

The addition of thyroxine (3, 5, 3′, 5′-*dl*-tetraiodothyronine) or triiodothyronine (3, 5, 3′-*l*-triiodothyronine) varying quantities to buffered media containing hemidiaphragms or liver slices from normal rats produced no acceleration in oxygen uptake of these tissues incubated for periods up to eleven hours. Hypothyroid rats were injected daily for ten days with 0.3 μM *l*-thyroxine or *l*-triiodothyronine; on the eleventh day hemidiaphragms and liver slices were incubated in Krebs-Ringer phosphate medium at

pH 7.4, and were found to consume significantly more oxygen per hour than similar tissues removed from a comparable group of animals treated only with saline. Moreover, the tissues of the rats given triiodothyronine exhibited a greater utilization of oxygen than those from the animals which received thyroxine. In another experiment the oxygen consumption of a rat-heart homogenatecytochrome *c* system with succinate as substrate at pH 7.8 was augmented by the direct addition to the medium of equimolar amounts of either *l*-thyroxine or *l*-triiodothyronine. However, the rate of oxygen uptake increased more rapidly, reached a higher point, and returned to the control level faster in the presence of triiodothyronine than in the presence of thyroxine. Thus triiodothyronine, like thyroxine, is not effective in stimulating oxygen consumption when added directly to intact tissues incubated *in vitro*. Yet triiodothyronine is more potent than thyroxine in accelerating the oxygen utilization of 1) tissues from animals injected with these compounds, and 2) a specific rat-heart homogenate system to which these compounds are added.

43. Comparative Calorigenic Effect of *l*-Triiodothyronine and *l*-Thyroxine Given Intravenously to Myxedematous Patients.

Charles M. Blackburn, M.D. (by invitation), *William M. McConahey, M.D., F. Raymond Keating, Jr., M.D.* and *A. Albert, M.D.*

From Mayo Clinic, Rochester, Minnesota.

The effects of *l*-triiodothyronine and *l*-thyroxine given intravenously have been compared in 6 patients with myxedema. Each patient received one to four injections over a period of several months. The basal metabolic rate of each patient was obtained daily for one to two weeks before injection, for training and to establish a base line, and for three to seven weeks after each injection. Significant qualitative differences were observed in the calorigenic response to each drug. The maximal response to *l*-triiodothyronine occurred in twenty-four to forty-eight hours and was two to three times as great as the maximal response to thyroxine. The maximal response to thyroxine occurred in seven to ten days. Also, the initial response to *l*-triiodothyronine appeared sooner. The biologic decay rate of the two substances appeared to be of similar order and possibly identical. Because of the foregoing, the only valid basis for quantitative comparison between the two substances which could be found was the area under each curve, which measures total calories contributed by each dose to the base-line level. Measured in this way, the calorigenic effects appeared to be substantially the same and possibly identical.

44. A Unique Serum Iodine Component in Certain Patients with Thyroid Carcinoma.

Jacob Robbins, M.D. (by invitation), *J. E. Rall, M.D.* and *Rulon W. Rawson, M.D.*

From the Memorial Center for Cancer and Allied Diseases, New York, N. Y.

The serum iodine elaborated by functioning thyroid carcinoma has been studied in 15 patients. We have previously reported that this did not differ from the secretion of normal or hyperplastic thyroid glands unless intensive irradiation released unchanged thyroglobulin. In 2 recently studied patients, however, the serum contained large amounts of a different iodine-containing substance.

Detailed studies of serum I^{131} in 1 patient, at intervals up to twenty-eight days following the administration of 250 millicuries of I^{131} revealed that: 1) it was partially soluble in *n*-butanol (40 per cent of the total), 2) it was entirely precipitable with the serum protein, 3) it did not move from the origin in filter paper chromatography, 4) it was 90

per cent soluble in 1.75 M phosphate buffer (pH 6.5) and precipitated sharply between 2.28 M and 2.80 M, 5) it migrated with the serum albumin during zone electrophoresis (pH 8.6), 6) in the preparative ultracentrifuge it sedimented at a rate close to that of albumin, and 7) chromatography of alkaline hydrolysates revealed the presence of two iodine-containing components of uncertain identity.

These criteria clearly differentiated this material from the "normal" thyroxine-protein complex, from thyroglobulin and from triiodothyronine.

45. Effect of Concurrent Dibenzyline Administration on Thyroxine-Induced and Epinephrine-Induced Increased Oxygen Consumption.

Dorsey E. Holtkamp, Ph.D. and *Arthur E. Heming, Ph.D.*

From the Research and Development Division, Smith, Kline & French Laboratories, Philadelphia, Pa.

Compared with the mean control level of 7.0 L./sq. M./hr., subcutaneously administered doses of 0.1, 0.2, and 0.5 mg./Kg./day of thyroxine (*l*-thyroxine sodium pentahydrate) for three days were found to increase the oxygen consumption of fasted, young adult rats (6 per group) to levels of 7.4, 8.3, and 8.5 L./sq. M./hr., respectively. Dibenzyline (*n*-phenoxyisopropyl-*n*-benzyl-β-chlorethylamine hydrochloride, SKF No. 688-A) administered in the food at a level of 0.025 per cent during the first two days of thyroxine treatment, inhibited this increase by 104 per cent (P, 0.01 by t test), 32 per cent, (P, 0.05; 0.02) and 23 per cent (P, 0.05), respectively. When tests were repeated in adrenalectomized rats using 0.2 mg. thyroxine/Kg./day and 0.025 per cent Dibenzyline, the inhibition amounted to 42 per cent (P, 0.05; 0.02). Moreover, at the 0.025 per cent level in the diet, Dibenzyline inhibited to the extent of 71 per cent (P, 0.01), the increase in oxygen consumption normally elicited by 0.1 ml./Kg. of subcutaneously administered epinephrine ("Adrenalin" in oil, 1:500 suspension). In control tests, using the same level of Dibenzyline alone, the compound had no effect on the oxygen consumption. Dibenzyline, therefore, in some as yet unexplained fashion, appears to be able to block both the thyroxine-induced and epinephrine-induced increased oxygen consumption of rats.

46. Influence of Cortisone and Desoxycorticosterone Upon the Renal and Thyroidal Metabolism of Iodine.

Sidney H. Ingbar, M.D. and *Bruce F. Chandler, M.D.* (introduced by Laurence H. Kyle, M.D.)

From the Department of Biophysics, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

Cortisone, when administered to hypophysectomized rats, depresses the thyroidal response to exogenous TSH, as measured by the uptake of I^{131}. In attempting to elucidate the origins of this action of cortisone, we have found that the rate of clearance of plasma iodide by the thyroid glands of hypophysectomized rats is unaltered by cortisone, regardless of whether TSH is also administered. The decreased uptake of radioactive iodine, which occurred in hypophysectomized rats given cortisone, was entirely explained by a more rapid loss of I^{131} from the plasma. This augmented disappearance rate was found to be the result of a marked increase in the renal clearance of iodide.

Adrenalectomy, unlike hypophysectomy, did not decrease the renal clearance of iodide. Cortisone was found to augment, and DCA to diminish the clearance of iodide by the kidneys of adrenalectomized rats. It therefore appeared that the 11-17-oxygenated

and the 11-desoxy-like steroids act antagonistically upon the renal excretion of iodide. Since, in the rat, secretion of 11-desoxy-like hormones continues after hypophysectomy, it is suggested that the decreased renal clearance of iodide found in these animals may have resulted from the unopposed action of DCA-like steroids. The administration of cortisone would then restore the balance toward normal. In the adrenalectomized rat, both antagonistic factors being absent, the renal clearance of iodide approaches that of the normal animal.

47. The Effect of Sodium Iodide, Thyroxine and Cortisone on Serum TSH Levels in Thyroidectomized Rats.

Benjamin Simkin, M.D., Paul Starr, M.D. and *Charles Hancock, B.S.* (by invitation).

From the University of Southern California School of Medicine, Los Angeles, California.

Various groups of thyroidectomized adult rats received injections of either sodium iodide, thyroxine or cortisone, in order to evaluate the effect of each of these substances on the elevated seru n TSH levels of these animals. TSH assays of rat sera were performed by our modification of D'Angelo's stasis tadpole method. If the increase in mean acinar cell height (MACH) from the sera of untreated thyroidectomized rats be taken to represent 100 per cent, then the effect of treatment with the foregoing substances may be tabulated as follows:

Duration of treatment	Thyroidectomized rats					Untreated normal rats (intact thyroid)
	Untreated	NaI (4 mg./day i.p.)	Thyroxine (3μgm./100 Gm. body wt./day i.m.)	Cortisone (5 mg./day i.m.)	Cortisone (25 mg./day i.m.)	
1 day	100%	92%	60%	109%	207%	36%
2 days	100	109	35	138	130	38
5 days	100	99	7	—	—	17
7 days	100	—	—	55	60	45
10 days	100	68	18	—	—	39

The administration of NaI caused no significant change in serum TSH levels, despite very high serum inorganic iodide concentrations of 219 to 1,220 micrograms per 100 ml. Thyroxine administration resulted in prompt significant suppression of serum TSH. Cortisone at both dosage levels produced no clear cut change in serum TSH concentration, although there is a suggestion of an increase in serum TSH at one and two days, and partial suppression at seven days.

48. The Influence of Cortisone on Pituitary-Thyroid Interaction in Normal and Goitrous Rats.

S. A. D'Angelo, Ph.D., C. E. Stevens, Ph.D. (by invitation), *K. E. Paschkis, M.D.* and *A. Cantarow, M.D.*

From the Jefferson Medical College, Philadelphia, Pa.

The effect of cortisone on pituitary-thyroid interplay was investigated in normal and in goitrous rats by determining thyrotropic hormone (TSH) levels in serum and in the adenohypophysis after subcutaneous administration of the steroid (2.5–5.0 mg. daily, for nine to eighteen days). Normal animals receiving cortisone showed no significant difference in thyroid-body weight ratio or in TSH content of blood and pituitary, compared with values of pair-fed controls. Blood TSH titers were slightly diminished when compared to those in rats fed "ad libitum." The administration of cortisone to animals fed propylthiouracil in the ration (0.05 per cent) produced greater thyroid hyperplasia and higher TSH blood titers than in pair-fed animals receiving goitrogen alone. In both groups, thyroidal I^{131} uptakes and TSH stores in the pituitary were decreased substantially.

The results suggest that the influence of adrenocortical steroids on the pituitary-thyroid mechanism is conditioned by the level of thyroid activity itself.

49. Observations on the Control of Thyrotropin Secretion of Intraocular Pituitary Implants in Hypophysectomized Mice.

Robert O. Scow, M.D. (by invitation) and *Monte A. Greer, M.D.*

From the NIAMD and NCI, National Institutes of Health, Bethesda, Md.

Previous experiments showed that the thyroids of hypophysectomized mice bearing intraocular pituitary implants accumulated in four hours 20–30 times as much I^{131} per unit of thyroid weight as the thyroids of hypophysectomized mice and $\frac{2}{3}$ as much as those of intact controls. After a single injection of propylthiouracil, the thyroid/serum I^{131} ratio (T/S) averaged 175 in implanted-hypophysectomized, 30 in hypophysectomized, and 265 in intact animals. Thyroid weights were the same in implanted and in non-implanted hypophysectomized mice.

Daily injections of 5 μg. of thyroxine in two weeks reduced the four-hour I^{131} accumulation in the thyroid and the T/S ratio in both intact and implanted-hypophysectomized animals to the hypophysectomized mouse levels. The T/S ratio was increased from two to fourfold in implanted-hypophysectomized mice when fed 0.3 per cent propylthiouracil for three weeks, whereas that of intact controls was only slightly increased; the increased T/S ratio averaged 50–100 per cent higher in implanted than in similarly treated intact mice. The thyroid weight was increased threefold in propylthiouracil-fed intact controls, whereas it was only slightly increased in implanted-hypophysectomized mice. Propylthiouracil feeding had no effect on T/S ratio or thyroid weight in hypophysectomized mice.

These findings suggest that the blood thyroxine level can markedly affect thyroid I^{131} uptake and T/S ratio in hypophysectomized mice bearing intraocularly implanted pituitaries, whereas its effect on thyroid weight is slight.

50. The Diagnosis of Equivocal Hypothyroidism, Using Thyrotropic Hormone (TSH).

Norman G. Schneeberg, M.D., William H. Perloff, M.D. and *Lester M. Levy, M.D.*

From the Endocrine Clinic, Philadelphia General Hospital, Philadelphia, Pa.

Thyrotropic hormone (TSH) increases the thyroid uptake of radioiodine in euthyroidism and in pituitary myxedema but not in primary thyroid myxedema. Consequently, two weeks following a baseline I^{131} uptake, TSH was administered to 66 patients, (14 of whom were instances of doubtful or "borderline" hypothyroidism), and the up-

take repeated. The final diagnoses were made without reference to the results of the TSH test. In groups F and G (see Table) final diagnoses were made only after one year or more of observation, repeated BMR and cholesterol determinations and several trials of desiccated thyroid. Results were as follows:

Final diagnosis	No. of cases	Percentage increase of I^{131} uptake above baseline	
		Mean	Range
A. Post-thyroidectomy myxedema	5	+ 5	− 6 to +11
B. Myxedema and cretinism	16	+ 2	− 8 to + 5
C. Pituitary myxedema	10	+33	+20 to +48
D. Euthyroidism (initial I^{131} uptake below 15%)	6	+29	+15 to +37
E. Euthyroidism (initial I^{131} uptake above 15%)	15	+18	+ 6 to +35
F. Suspected hypothyroidism (later proved)	5	+ 2	− 2 to + 8
G. Suspected hypothyroidism (later proved to be euthyroidism)	9	+17	0 to +37

The test failed to corroborate the final diagnosis in 1 patient of group G, but in this instance the baseline uptake was 22 per cent. The results indicate that TSH is a useful diagnostic adjunct in the evaluation of questionable hypothyroidism.

51. The Tolerance of Patients with Myxedema to Exogenous Thyroid Substance.

Richard Goldsmith, M.D. (by invitation) and *John B. Stanbury, M.D.*

From the Thyroid Clinic of the Massachusetts General Hospital, Boston, Massachusetts, and the Department of Internal Medicine of the Cincinnati General Hospital, Cincinnati, Ohio.

Eleven patients with clinical and laboratory myxedema were studied. Baseline weight, BMR and serum-PBI studies were obtained. Then each patient was started on thyroid substance by mouth. The original dosage was usually 32 mg. daily and was doubled at two-week or three-week intervals. The foregoing data were accumulated at the end of each dosage period. In most instances this program was continued until the patient became minimally hyperthyroid. Four subjects had spontaneous myxedema and in 7 subjects the disease developed following surgical thyroidectomy. Eight of these 11 patients required an average of 0.8 gram of thyroid substance daily before showing clinical evidence of drug toxicity. The BMR averaged +19 per cent and the serum PBI level averaged 12.0 micrograms per 100 cc. at the time toxicity was apparent. The remaining 3 subjects tolerated an average of 0.3 gram of thyroid daily without any evidence of hyperthyroidism. Three euthyroid subjects were also studied for comparison. An excellent paper by Farquharson *et al.* (*Ann. Int. Med.* **34**:1008, 1951) has been published presenting similar data on a large series of euthyroid subjects. About 0.5 gram of exogenous thyroid substance daily was required to produce drug toxicity in the euthyroid individuals. This occurred when the BMR was in the range of +13 per cent, with serum PBI concentrations averaging 10.2 micrograms per 100 cc. The implications of these findings will be discussed, particularly with reference to current concepts of end-organ responsiveness in the myxedematous subject.

TO BE PRESENTED IF TIME PERMITS

52. The Effects of Cortisone in Acute Diffuse Thyroiditis.

Robert M. Salassa, M.D. (introduced by Randall G. Sprague, M.D.)

From the Division of Medicine, Mayo Clinic, Rochester, Minnesota.

Cortisone was administered orally to 24 patients who had acute diffuse thyroiditis. The administration of 100 to 200 mg. of cortisone daily resulted in prompt and consistent suppression of fever and pain. The tenderness of the thyroid gland disappeared, and there was a rapid decrease in the sedimentation rate. However, as the dose of cortisone was reduced to 50 mg. per day or less, there was a return of symptoms and an increase in the sedimentation rate. The symptoms again disappeared when the dose of cortisone was increased. The shortest period of therapy was nine days and the longest period was six months, with an average of approximately eight weeks. Cortisone is useful as a form of symptomatic therapy in the management of severe acute diffuse thyroiditis, although it does not appear either to cure the disease or to shorten its course.

53. Investigation of the Extrathyroidal Effects of Thyroid-Stimulating Hormone.

L. H. Kyle, M.D. and (by invitation) *Capt. W. C. Welham, M.C., U.S.N., Lt. P. D. Doolan, M.C., U.S.N.* and *M. Schaaf, M.D.*

From the Department of Medicine, Georgetown University School of Medicine and the Experimental Diving Unit, U. S. Naval Gun Factory, Washington, D. C.

Despite frequent reference to the extrathyroidal effects of thyrotropic hormone (TSH), especially with respect to fat and water, no adequate proof of such tissue-affecting action has been furnished. Two patients with primary myxedema were studied by the usual metabolic procedures and by repeated measurement of body composition, using the specific gravity technique, before and during the administration of TSH. This method of estimating body composition, which is dependent upon determination of equivalent volume by hydrostatic weighing, revealed no significant change in body fat, water or fat-free solids during administration of TSH for respective periods of twenty-two and twenty-four days. In neither patient was there any change in the serum lipid partition, in blood levels of pyruvic acid and amino-nitrogen, or in urinary nitrogen excretion. In 1 patient, measurement of sodium and potassium excretion revealed no significant variation and the blood ketone levels remained constant throughout the administration of TSH. Following treatment with thyroid hormone there occurred a significant change in body composition, with loss of all three major body constituents, and a decrease in blood lipids, involving both neutral fat and cholesterol. From the results of this study it appears probable that TSH can exert a marked metabolic effect only through stimulation of functioning thyroid tissue.

54. Behavior Cycles Produced in Rats by Thyroidectomy, Injection of I^{131} or by Feeding Sulfamerazine.

Curt P. Richter, Ph.D., G. E. Seegar Jones, M.D. and *James W. Woods, B.A.* (by invitation).

From the Psychobiological Laboratory, Johns Hopkins Hospital, Baltimore, Md.

Regular cycles in spontaneous running activity, food intake, body weight and the

reproductive tract were found in 2 out of 14 thyroidectomized rats, 3 out of 4 rats treated with I^{131}, and 4 out of 16 rats fed sulfamerazine (0.5–2.0 per cent in food for one hundred days).

Each cycle consisted of two phases, one in which the rats were very active, the other in which they were almost totally inactive. When they first manifested themselves, the cycles lasted from thirty to fifty days; they then became progressively shorter, finally reaching a constant length near twenty days.

During the active phase of the cycle the 4-day to 5-day estrous and vaginal smear cycles were present and superimposed on the longer cycles; during the inactive phase the estrous and vaginal smear cycles were absent. During the active phase the rats ate much less food and lost weight; during the inactive phase they ate large amounts of food and gained weight.

After thyroidectomy the cycles appeared only when a very small but definite amount of tissue remained; the cycles did not appear when either too much or too little thyroid tissue was left. It is likely that after treatment with I^{131} or sulfamerazine their appearance likewise depends on leaving a small amount of active tissue. The operation of a feed-back mechanism between the anterior lobe of the pituitary and the small remnant of thyroid tissue may account for the appearance of the cycles.

SATURDAY, MAY 30, 1953

9:00 A.M. HYPOTHALAMUS, PARATHYROID, GROWTH HORMONE AND PANCREAS

CHAIRMEN: FRANCIS D. W. LUKENS AND JAMES H. LEATHEM

55. Endocrine and Other Clinical Manifestations of Hypothalamic Disease; a Survey of 60 Cases with Autopsies.

Hans G. Bauer, M.D.

From the New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York, N. Y.

Sixty cases with neoplastic, inflammatory and other pathology in the hypothalamus, in which autopsies were performed, have been evaluated statistically as to the incidence of symptomatology and laboratory abnormalities. A high incidence of involvement of gonadal function was evident. Twenty-four patients suffered from precocious puberty, and 19 from depressed sexual function. There was corroborating statistical evidence that precocious puberty is caused by a pathologic process in the posterior hypothalamus, requiring an intact anterior pituitary; whereas hypogenitalism results from lesions in the infundibulotuberal region, with or without an intact pituitary. One case of each variety, with pinpoint lesions in the "gonadal centers," is described. The frequency of occurrence of emaciation, obesity, somnolence, diabetes insipidus and combinations of these disorders is discussed. Basal metabolic rates were found to be low, with 1 exception (a normal rate). In each case, when corresponding blood cholesterol levels were given, they were found to be proportionately low. Glucose tolerance tests in 5 of 10 patients, were abnormal; but in urinary studies of 59 subjects no disturbance of carbohydrate metabolism was uncovered. This indicates the importance of performing the glucose tolerance test in all of these subjects. Among other manifestations evaluated are dyshidrosis, anorexia, bulimia, urinary incontinence and dissociation of temperature, pulse and respiration.

56. Fractionation of Hypothalamus for Pituitary-Stimulating Activity.

M. A. Slusher, Ph.D. (by invitation) and *Sidney Roberts, Ph.D.*

From the Department of Anatomy and Physiological Chemistry, School of Medicine, University of California at Los Angeles, and the Department of Investigative Medicine, Veterans Administration Hospital, Long Beach, California.

Aqueous and lipid extracts of various areas of beef brain have been prepared and subfractionated. The pituitary-stimulating activity of the fractions obtained has been assayed by several procedures. These include observation of the eosinopenia and adrenal ascorbic-acid decline after injection into intact rats and measurement of the alteration in metabolism of isolated rat pituitary tissue after addition *in vitro*. Thus far, the presence of at least two active fractions is indicated. These are: 1) a water-soluble, nonprotein material present in all parts of the brain tested, and 2) a lipid-soluble material apparently present only in hypothalamic areas. Further work is in progress which is directed toward the isolation and characterization of the active substances.

57. Antidiuretic Hormone Antagonism.

George E. Miller, M.D. and *Charles E. Townsend, M.D.* (introduced by George F. Koepf, M.D.)

From the University of Buffalo School of Medicine and the Buffalo General Hospital, Buffalo, N. Y.

The trained water-loaded dog responds with a characteristic reduction in urine flow to minute amounts of Pitressin administered intravenously. This effect is reproducible within fairly narrow limits over a long period of time. The response of 2 such animals to 1- and 2-milliunit amounts of Pitressin was studied before and after a one-month period, during which they received repeated intravenous injections of a rat-liver homogenate suspended in saline and incubated with 100 milliunits of Pitressin/0.1 Gm. of liver tissue. The *mean* change in minute urine volume (calculated from five-minute collection periods) following hormone administration is reported as the percentage of minute volume during a control period.

Dog A:	1 milliunit	before	−45%
		after	−15%
	2 milliunits	before	−70%
		after	−29%
Dog B:	1 milliunit	before	−28%
		after	+ 4%
	2 milliunits	before	−49%
		after	+ 1%

During any single experiment, testing several levels of ADH, this reduced responsiveness followed a regular pattern: sensitivity to the initial ADH injection was manifestly greater than to those which followed.

Three possible explanations of these observations have been considered: 1) increased rate of destruction of ADH; 2) production of a circulating antihormone; and 3) a local

cellular dysfunction at the site of ADH effect in the renal tubule. Although the evidence at this moment seems to favor the third possibility, studies designed to elucidate this problem are in progress.

58. Idiopathic Hypercalciuria.

Fuller Albright, M.D., Philip Henneman, M.D., Patricia H. Benedict, M.D. and *Anne P. Forbes, M.D.*

From the Stone Clinic of the Massachusetts General Hospital, Boston, Mass.

Twelve patients are presented illustrating a syndrome consisting of a normal serum calcium level, a low serum phosphorus level, increased urinary calcium excretion and, in most instances, Staphylococcus albus pyelonephritis, past or present. We have termed the condition idiopathic hypercalciuria. This dislocation of homeostasis has been brought to our attention because of the frequency of kidney stones as a complication.

Renal stones from these patients are composed consistently of calcium phosphate and calcium oxalate. The absence of magnesium and ammonium supports the suggestion that they are due to hypercalciuria per se and do not arise in the alkaline urine of a urea-splitting infection.

That this syndrome is not hyperparathyroidism is shown by the normal parathyroid histologic pattern seen upon exploration; that it is not a mild form of renal tubular acidosis, by the failure of urinary calcium to decrease on alkali therapy. A case is cited in which a patient with a low serum calcium level due to hypoparathyroidism showed severe hypercalciuria during a staphylococcus pyelonephritis; urinary calcium excretion fell during penicillin treatment.

The sequence of events is thought to be Staphylococcus albus pyelonephritis, renal tubular damage, diminished tubular reabsorption of calcium, hypercalciuria, tendency to hypocalcemia, compensatory hyperparathyroidism, and hypophosphatemia.

59. The Effect of Parathyroid Extract on Ammonium-Chloride-Decalcified-Bone Matrix and on the Serum Levels of Polysaccharides: Preliminary *in vivo* Studies in Parathyroidectomized Rats.

Claiborne L. Courtright, M.D. (by invitation), *M. R. Shetlar, Ph.D.* (by invitation) and *Edward C. Reifenstein, Jr., M.D.*, in collaboration with *Harry B. Jones, B.S.* and *R. C. McDougal, B.S.*

From the Section of Endocrinology and Metabolism, Oklahoma Medical Research Foundation; and the Departments of Biochemistry and Medicine, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma.

Recent evidence suggests that parathyroid hormone (PTH) has an effect on the uncalcified protein matrix of bone. It is not clear, however, whether PTH affects *a*calcified matrix, *de*calcified matrix, or both; or whether the hormone has any effect on calcified matrix.

The experiment herein reported was designed as an initial step toward determining the effect of PTH on *de*calcified matrix. Male adult Sprague-Dawley rats were parathyroidectomized, maintained on calcium and phosphorus intakes sufficient to give normal serum levels, then treated with ammonium chloride for six weeks, and finally with large doses of parathyroid extract for three days. Serum calcium and phosphorus levels were determined throughout the study. The experimental animals, and parathyroidectomized as well as parathyroidectomized-ammonium-chloride-treated control

animals, were sacrificed at the end of the study for bone morphologic studies. The bones were studied by histologic and histochemical techniques. The spectrum of serum polysaccharides was determined before and at the end of the experiment.

The serum polysaccharide levels showed a rise in the mucoprotein fraction upon administration of PTH. The findings in the bone specimens and detailed polysaccharide studies will be presented. The significance of these data will be discussed.

60. A Simple Test to Study Calcium Metabolism in Endocrine Disorders.

Herta Spencer, M.D., Isaac Lewin, M.D. and *Daniel Laszlo, M.D.* (introduced by Walter Fleischman, M.D.)

From the Division of Neoplastic Diseases, Montefiore Hospital, New York, N. Y.

The calcium tolerance test which is based on the 24-hour urinary calcium excretion following the slow infusion of a calcium test dose, has been applied to over 100 cases of metabolic and neoplastic bone disease. It has also been applied in the study of disorders of mineral metabolism of endocrine etiology, such as osteoporosis, Cushing's syndrome, hyperthyroidism and hypoparathyroidism. It revealed, in Cushing's syndrome, marked demineralization not detectable by roentgenograms or serum calcium levels. After removal of an adrenal adenoma, the reparative efforts could be readily demonstrated by this test, although they could not be appreciated by the usually employed laboratory aids. In hyperparathyroidism, the test revealed accelerated demineralization but it also indicated unimpaired utilization of calcium. Return to a normal pattern was noted after removal of a parathyroid adenoma. In hypoparathyroidism, the extreme tendency to retain calcium was shown by the test; the utilization of the administered dose was significantly higher than normal. The test has also been applied to 7 cases of senile osteoporosis. The simplicity and the sensitivity of the test for reflecting the response to therapy suggests its use in the study of derangements of mineral metabolism in endocrine disorders.

61. The Effect of "Pituitary Growth Hormone Preparations" in Human Subjects. A Summary of Quantitative Studies Extending Over a Four-Year Period.

Laurance W. Kinsell, M.D., Sheldon Margen, M.D., John W. Partridge, M.D., George D. Michaels, Ph.D., Harry E. Balch, M.D. and *John P. Jahn, M.D.*, with the technical assistance of *Marjorie Coelho, Eleanor Kipp* and *Florence Olson.*

From the Institute for Metabolic Research, Highland Alameda County Hospital, Oakland, California.

During the period 1948 to 1949 in this laboratory, two "highly purified growth hormone preparations," one prepared by Dr. Li, and one by Dr. Hays, and their respective associates, were evaluated by us in human subjects maintained on chemically constant diets. Both preparations caused pyrexia and resulted in a negative protein balance. Evaluation of similar or identical preparations by other investigators during the same period resulted in similar findings.

In 1950, a slightly different "growth hormone preparation" was evaluated in a patient with panhypopituitarism, and again resulted in a negative protein balance.

During 1951–1952, two growth hormone preparations were evaluated in a patient with little or no endogenous thyroid function. For the first time in our experience, a significant retention of nitrogen, potassium and phosphorus occurred in association with the administration of hormonal material of pituitary origin. There was no pyrexia. A

slight "rebound" appeared when the hormone was discontinued. In 1952, 3 patients received "pituitary growth hormone" prepared by Drs. Raben and Astwood, and by Drs. Hays and Steelman, respectively. Both lots of material were prepared by the "Raben method." The former material produced no significant effect at any dosage level used. The latter proved to be rather heavily contaminated with corticotropin, and gave the metabolic changes characteristic of such material.

From the foregoing, it seems probable that in one instance material of pituitary origin produced a significant anabolic effect in a patient with little or no endogenous thyroid activity. This may mean that thyrotropin contamination is in some measure responsible for the lack of anabolic effect of similar material administered to euthyroid patients.

62. Utilization of Labelled Glucose and Fructose by Liver Slices of Normal and Diabetic Rats.

Albert E. Renold, M.D., Frances B. Nesbett, A.M. and *A. Baird Hastings, Ph.D.* (introduced by George W. Thorn, M.D.)

From the Department of Biological Chemistry, Harvard Medical School, Boston, Mass.

Liver slices from normal, alloxan-diabetic and pancreatectomized rats were incubated with either glucose or fructose (30 mM./L.), both substrates being uniformly labelled with C^{14}. The ionic composition of the media used was designed to avoid significant changes in the intracellular cationic environment. Normal liver took up approximately three times more fructose than glucose (160, as compared to 60 micromoles per gram, per ninety minutes). However, most of the excess fructose taken up reappeared as glucose in the medium. The amounts of either glucose or fructose incorporated into glycogen were practically identical (31 and 35 micromoles per gram). Liver slices from alloxan-diabetic or pancreatectomized rats showed a 54 per cent decrease in glucose uptake and almost complete absence of glucose incorporation into glycogen (0.5 micromole per gram). Fructose uptake and conversion to glucose by the diabetic liver slices, however, were entirely normal, and the incorporation of fructose into glycogen averaged 8 micromoles per gram. Thus, although the by-passing of any difficulty in glucose-6-phosphate formation (by substitution of fructose for glucose) considerably increased hexose incorporation into glycogen by diabetic liver, the value reached was still only one fourth of the normal value for either glucose or fructose.

63. Metabolic Observations Following Total Pancreatectomy.

M. A. Ogryzlo, M.D. (by invitation), *A. G. Gornall, Ph.D.* (by invitation), *J. A. Dauphinee, M.D.* (by invitation) and *D. J. MacKenzie, M.D.* (by invitation) (introduced by R. F. Farquharson, M.D.)

From the Clinical Investigation Unit, Sunnybrook (DVA) Hospital, Toronto, Canada.

The patient, a 36-year-old woman, was subjected to a total pancreatectomy for organic hyperinsulinism, which was later shown to be due to surreptitious insulin administration. Opportunity was taken to determine the effects of pancreatin, insulin and cortisone on fat absorption, carbohydrate utilization, nitrogen metabolism, electrolyte balance and steroid hormone excretion in a case of true pancreatic diabetes. Fifteen grams of pancreatin were shown to be effective in increasing fat absorption, and largely relieved the symptoms of steatorrhea. The insulin requirement was approximately 50

units per day. Oral administration of cortisone during the uncontrolled diabetic state produced a further small but distinct rise in blood sugar concentration, associated with a negative nitrogen balance and a rapid increase in ketosis. When the patient was under good diabetic control, cortisone was found to antagonize the action of insulin in regard to carbohydrate and protein metabolism, resulting in a decreased utilization of glucose. The ratio of the antagonism for this patient appeared to be roughly 4 to 5 mg. of cortisone for each unit of insulin. There was little to indicate new glucose formation from fatty acid during cortisone administration. A study of the effects of growth hormone on the overall metabolic state is in progress.

64. The Physiologic Consequences of Selective Destruction of the Alpha Cells of the Pancreatic Islets.

Sidney S. Lazarus, M.D. (by invitation), *Bruno W. Volk, M.D.* (by invitation) and *Martin G. Goldner, M.D.*

From the Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, N. Y.

The discovery that cobaltous chloride causes selective damage or destruction of the alpha cells of the pancreatic islets in various animal species has opened a new approach to the study of islet function. This damage is observed within one hour after the intravenous administration of cobaltous chloride, and persists for several days. The administration of cobaltous chloride is also accompanied by an immediate and significant rise of the blood sugar level, which may persist for two to four hours. This is followed by a gradual return to pretreatment levels. Despite the persistent alpha-cell damage, normal glycemia is then maintained. During the period of demonstrable alpha-cell damage, new hyperglycemic episodes can be elicited in the same animal by repeated injections at brief intervals. In depancreatized animals, cobalt causes a similar rise in the blood sugar level. Cobalt has no lasting effect on the hyperglycemia of alloxanized animals. In view of these observations, it is presumed that the cobalt hyperglycemia is not dependent on the pancreatic alpha cells and that the physiologic role of these cells in blood sugar homeostasis is negligible.

65. Steroid Diabetes in Guinea Pigs and the Role of Insulin in the Adaptation to Cortisone Administration.

F. X. Hausberger, M.D. (introduced by W. H. Pearlman, M.D.)

From the Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia, Pa.

In guinea pigs (fed *ad lib.*) a steroid diabetes can be induced readily by 15 to 20 mg. of cortisone daily. There is an increased food consumption, an augmented gain of weight due to an increased fat deposition, and a marked glucosuria and hyperglycemia. The beta cells in the tremendously hypertrophic islets of the pancreas show a pronounced degranulation. The diabetes is maintained up to eighty days. It disappears slowly and body weight decreased temporarily after discontinuing cortisone administration. Studies on the effect of cortisone on the islets of partially pancreatectomized guinea pigs are in progress. An increased insulin secretion may be partly responsible for these metabolic effects of cortisone, resembling the situation in human hyperadrenocorticism (Cushing describes a hypertrophy of the islets in such patients). Therefore, normal and alloxan-diabetic rats were injected with cortisone, and with cortisone plus insulin, respectively. The results indicate that cortisone can induce an increased insulin secretion which, in

turn, causes a true growth of adipose tissue and an augmented fat deposition even while the body weight decreases. The role of a possibly increased insulin secretion in the adaptation syndrome following cortisone administration will be discussed.

TO BE PRESENTED IF TIME PERMITS

66. The Prediabetic Father.

W. P. U. Jackson, M.D. (introduced by Fuller Albright, M.D.)

From the Groote Schuur Hospital, Cape Town, South Africa.

In the prediabetic state, women who later have overt diabetes, are apt to produce large babies and stillbirths. In my series, over 60 per cent of such women claim babies who weighed more than 10 pounds at birth. The current most favored explanation ascribes the responsibility for this phenomenon to an excess of circulating growth hormone. There is little evidence for this, and much against it. Acromegalics, for instance, in a short personal series, do not appear to have a great tendency to produce babies of excessive size.

New evidence is presented concerning the children of diabetic *fathers*, which indicates that their birth-weights are above average, and the stillbirth rate is not increased. This suggests that the onus of production of large babies cannot be borne by maternal factors, but must in part be an inherited phenomenon, linked in some way to the diabetic genetic constitution of *either* parent. The concept of the "prediabetic father" is thus born.

2:00 P.M. JOINT SESSION WITH THE AMERICAN DIABETES ASSOCIATION

67. Growth Hormone and Carbohydrate Metabolism.

R. C. de Bodo and M. W. Sinkoff

From the Department of Pharmacology, New York University College of Medicine, New York, N. Y.

1. Adrenalectomized dogs, although insulin sensitive, are much less so than adrenalectomized-hypophysectomized dogs. The secondary hypoglycemia of the intravenous glucose tolerance test of adrenalectomized dogs is less marked than that of adrenalectomized-hypophysectomized dogs. These differences are not due to ACTH since administered ACTH produces no changes in the abnormal carbohydrate metabolism of adrenalectomized dogs. 2. The disturbed carbohydrate metabolism of adrenalectomized-gonadectomized dogs resembles that of adrenalectomized non-gonadectomized dogs. 3. Hypophysectomized dogs, treated with thyroxine or whole thyroid, show little change in their abnormal carbohydrate metabolism. 4. Growth hormone, 0.02–2.0 mg./Kg/day intramuscularly, ameliorates or abolishes the exaggerated insulin response and secondary hypoglycemia of the glucose tolerance test of hypophysectomized and adrenalectomized-hypophysectomized dogs, but invariably produces diabetes, insulin resistance and/or toxicity. These latter effects are eliminated when the growth hormone subsequently is administered to the steroid-treated hypophysectomized dog, thereby producing a normal carbohydrate metabolism. Conclusions: An anterior pituitary factor other than ACTH has a role in the regulation of normal carbohydrate metabolism. It is not thyrotropin or the gonadotropins; most probably it is the growth hormone. Although the adrenocortical steroids are not essential for the production of a growth-hormone effect in physiologic life, these two factors appear to exert their action in balance with each other.

68. Metabolic Effects of Crystalline Growth Hormone (Somatropin) in Man.

Ephraim Shorr, M.D., Anne C. Carter, M.D., Byrl J. Kennedy, M.D. (by invitation) and *Richmond W. Smith, Jr., M.D.* (by invitation).

From the Department of Medicine, Cornell University Medical College and The New York Hospital, New York, N. Y.

During the past five years, metabolic balance studies have been carried out with 9 different lots of a crystalline growth hormone (Somatropin) prepared by the Armour Research Laboratories according to the method of Wilhelmi, and with one lot prepared by Raben and Westermeyer. The Armour preparation was free of ACTH but contained TSH in concentrations of 0.02–0.08 USP units per mg. The subjects were 2 girls and 3 boys of abnormally short stature. Primary amenorrhea and thyroid insufficiency of pituitary origin were present in one of the girls; the latter defect was corrected by administration of desiccated thyroid.

Growth hormone was administered over periods of from two to eleven weeks, usually in ascending doses up to a maximum of 300 mg. daily. Significant storage of nitrogen, calcium and phosphorus occurred in both girls while receiving several lots of Somatropin. One became glycosuric after 9,375 mg. given over eleven weeks; and both showed diabetic sugar-tolerance curves and increased insulin resistance, with a return to normal values after cessation of therapy. Administration of the Raben-Westermeyer preparation for sixteen days (total 1,700 mg.) in one of the girls was without anabolic or carbohydrate effects. No significant metabolic alterations were observed in the 3 boys during Somatropin administration. Additional data relating to other metabolic functions will be described.

69. The Banting Memorial Lecture: An Interpretation of Diabetes in Light of its Pathology.

Shields Warren, M.D. (by invitation).

(No abstract.)

70. Effect of Cortisone on Carbohydrate Metabolism, Measured by the Glucose Assimilation Coefficient.

P. A. Bastenie, M.D. (by invitation), *J. R. M. Franckson, M.D.* (by invitation) and *V. Conard, M.D.* (by invitation) (introduced by E. H. Rynearson, M.D.)

From the Medical Clinic, Brugmann Hospital, Brussels, Belgium.

After a short phase of diffusion from the blood into the extracellular fluids, the disappearance rate of intravenously injected glucose depends on its entry into the cells. The values of blood glucose obtained between fifteen and sixty minutes draw an exponential curve. Mathematical analysis of its semi-logarithmic representation yields a factor K which at any time between these limits commands the "glucose assimilation" (Conard *et al.:* (in press) *Arch. internat. de pharmacodyn. et de therap.*).

In 18 patients submitted to cortisone treatment, K (glucose assimilation coefficient) was before treatment: 1.84 0.7; after seven days (1 Gm.): 1.22 0.39; after twenty-one days (3 Gm.) 1.69 0.56; after one hundred days (10 Gm.): 2.18 0.34. Statistical analysis, using Student's *t* test and pairing, shows that these variations are significant.

Whereas it is generally admitted that usual therapeutic doses of cortisone have no

definite effect on glucose metabolism of normal subjects, calculation of the glucose assimilation coefficient shows that under this therapy, there is at first a marked depression, later a restoration to normal, and still later an improvement of the glucose assimilation rate.

These effects may be due at first to reduced insulin activity, and later to inhibition of endogenous steroid secretion.

71. Studies in Experimental Diabetic Acidosis: Comparison of the Effect of Fructose and Glucose in the Initial Hours of Treatment.

Max Miller, M.D., J. R. Murphy, M.D., J. W. Craig, M.D. and *Hiram Woodward, Jr., B.S.*

From the Department of Medicine, Western Reserve University, Cleveland Ohio.

Diabetic acidosis was induced in a human volunteer by withdrawal of insulin on four separate occasions. Complete balance data were obtained during the withdrawal period and during treatment. These included determinations of sodium, potassium, chloride, phosphate, nitrogen, ketone, carbohydrate and water. During the withdrawal period on three occasions the subject excreted the ingested carbohydrate almost quantitatively, the D:N ratios being 3.8, 2.5 and 3.1, respectively. On the fourth occasion, in the absence of insulin, approximately 150 grams of intravenously administered fructose ($\frac{1}{5}$ of the amount given) were shown to be utilized.

The treatment-period studies were designed to test rigidly the effect of fructose and glucose during the first six hours. The amount and time of administration of insulin was the same in each of the three experiments. Similarly, total fluid and grams of sodium chloride given were kept constant. In the first study, 175 grams of fructose were given during the six hours; in the second, 175 grams of glucose were given in an identical manner, and in the third experiment no carbohydrate was administered. It was found that fructose greatly enhanced the amount of carbohydrate utilized, without increasing significantly the amount of water, sodium or potassium excretion in the urine. The effects on ketone and protein metabolism will also be presented.

72. The Serum Polysaccharides in Diabetic Patients With and Without Degenerative Vascular Disease.

Harold Rifkin, M.D., James Berkman, M.D. and *George Ross, B.S.*

From the Medical and Laboratory Divisions, Montefiore Hospital, New York, N. Y.

There has been increasing speculation that increased concentrations of circulating glycoproteins may be intimately associated with the development of degenerative vascular complications of diabetes mellitus. Although scattered data bearing on this problem are available, alterations of the serum glycoproteins in diabetic patients have not been systematically investigated. This report is based on a comparative study of certain protein-bound carbohydrate fractions in the sera of 66 diabetic patients with and without degenerative vascular disease. In view of possible effects of renal dysfunction on these fractions, the "complicated" diabetic patients have been divided into two groups, depending upon the presence or absence of hypertension, proteinuria, and renal insufficiency. Fifteen patients with neither diabetes nor renal disease formed a control group.

The fractions studied included 1) total nonglucosamine polysaccharide bound to a serum protein (method of Graff *et al.*), 2) serum glucosamine (West), 3) protein and

tyrosine content of the serum mucoprotein (Winzler *et al.*), and 4) polysaccharide content of the serum mucoprotein fraction (Graff).

Our results reveal the following:

1. The concentrations of total polysaccharide, glucosamine, and both protein (tyrosine) and polysaccharide components of the mucoprotein fraction are within normal limits in the serum of diabetic patients with degenerative vascular disease.

2. In diabetic patients with degenerative vascular disease, the total serum polysaccharide and glucosamine are *distinctly elevated.* Moreover, the values in such patients are not significantly influenced by the presence of renal dysfunction. In contrast, the mean value of both the carbohydrate and tyrosine components of the serum mucoprotein remain approximately within normal limits.

3. A direct correlation between blood sugar levels and serum polysaccharides is not found.

The significance of these findings and their relationship to the pathogenesis of degenerative vascular complications of diabetes mellitus will be discussed.

73. Spontaneous Diabetes in Dogs: An Account of 8 Cases.

Henry T. Ricketts, M.D.

(No abstract.)

READ BY TITLE

74. Study of the Morphologic and Histochemical Changes Produced by Estrogens in Adult Human Testes.

F. A. de la Balze, M.D. (by invitation), *R. E. Mancini, M.D.* (by invitation), *G. E. Burr, M.D.* and *Juan Irazu, M.D.* (by invitation).

III Catedra de Clinica Medica, Facultad de Medicina, Buenos Aires, Argentina.

Testicular biopsies were performed in 15 patients with prostatic carcinoma, both before, and 8, 10, 11, 13, 23, 25, 32, 33, 35, 40, 45, 60, 69, 70 and 71 days after administering stilbestrol in daily doses of from 5 to 50 mg.

The observed histologic lesions are described, in the order of their appearance.

1. Initially there was a proliferation of juvenile fibroblasts in the tunica propria; the fibroblasts had lost their normal histochemical features; there was slight reticular fibrosis, and atrophy of the germinal epithelium.

2. Then there was an increase of the tubular fibrosis; collagenous fibers predominated; fibroblasts increased; there was thickening of the basal membrane, marked atrophy of the germinal epithelium, decrease of trophic Sertoli cells, and involution of most of the Leydig cells.

3. Later, there were fewer fibroblasts in the tubular wall, intense fibrosis, hyalinosis and atrophy of the basal membrane, and atrophy of the Sertoli cells. In the intertubular connective tissue there was involution of all the Leydig cells and proliferation of juvenile fibroblasts.

It is suggested that:

a) Fibrosis of the tubular wall is the earliest and most important change; intratubular changes are partly secondary.

b) The changes in the tubular wall are a consequence of abnormality of the fibroblasts; the latter, as well as the change in the Leydig cells, may be induced by changes in the pituitary gonadotropins produced by the estrogens.

75. The Control of Functional Uterine Bleeding (Menometrorrhagia) by the Oral Administration of Progesterone and Methyltestosterone.

Meyer H. G. Blatt, M.D. (by invitation), *Hans Wiesbader, M.D.* (by invitation) and *Herbert S. Kupperman, M.D., Ph.D.*

From the Departments of Therapeutics and Obstetrics and Gynecology, New York University College of Medicine, and the Obstetrical and Gynecological Service of the Third (New York University) Surgical Division, Bellevue Hospital, New York, N. Y.

The control of excessive uterine bleeding in the menarche and in young adolescent and adult females has many times been managed by diagnostic dilation and curettage. More recently a "medical curettage" achieved by the parenteral use of progesterone, administered either alone or in combination with testosterone, has been found to be a very valuable substitute for the time-honored "D & C" in the therapy of menometrorrhagia. To overcome the inconvenience of parenteral therapy with progesterone, 45 patients with functional uterine bleeding were treated by the oral administration of a combined methyltestosterone and progesterone tablet. The tablet contained 50 mg. of each steroid and was administered four times per day for five days. Prior to the administration of the medication the endocrine status and endometrial pattern of the patient were studied by appropriate measures. Oral therapy induced a cessation of menstrual bleeding within one to three days after onset of therapy. Withdrawal bleeding, or the so-called "medical curettage," occurred within one to five days after the last pill had been ingested. Endometrial biopsy specimens taken before initiating therapy and on the first day of withdrawal bleeding demonstrated that this dose of oral progesterone was capable of changing a cystic glandular hyperplastic or proliferative endometrium noted in most of our patients into a secretory-phase endometrium. The total dose of 1,000 mg. of methyltestosterone and 1,000 mg. of progesterone did not produce any untoward effect. The androgen was not repeated in subsequent periods. Hence, no arrhenomimetic phenomena were noted. Cyclic initiation of menstrual cycles in these patients was attained later by the administration of progesterone alone, either orally or in the form of a single injection containing 100 mg. of the steroid. Either form of therapy was administered four weeks after the patients had taken their first tablet for excessive uterine bleeding. The regimen described here was comparable in effect to parenteral therapy and was successfully used in patients with functional uterine bleeding. The oral method had the added convenience of avoiding parenteral medication and permitted easier control of abnormal uterine bleeding.

76. The Time of Ovulation in the Human Female. V. Transvaginal Pelvioscopy: Correlation of Ovarian Status with Basal Body Temperature Curve and Endometrial Biopsy Findings.

A. R. Abarbanel, M.D.

From the Department of Obstetrics and Gynecology, College of Medical Evangelists, Los Angeles, California.

In 200 women, the status of the follicular apparatus, determined by pelvioscopy, has been carefully correlated with the basal body temperature (B.B.T.) curve and with endometrial biopsy findings. With regard to the B.B.T. curve, the low point (L.P.) before the sustained rise of 0.4° F. or more, for at least twelve days, was considered as the refer-

ence point. From 5 to 20 observations were made on each day of the cycle, from seven days before the low point (L.P. minus seven) through L.P. plus fourteen.

The large ripening follicle of that cycle was apparent in about 25 per cent at L.P. minus five, and in about 60 per cent at L.P. minus two. On this day and the next, 30 per cent disclosed a pre-ovulatory follicle. On the day of the low point, 22 per cent were found to have an early postovulatory follicle; another 22 per cent had a large preovulatory follicle; and 44 per cent showed just a large ripe follicle. In the course of the next three days, the percentage with an early corpus luteum rose from 22 per cent on L.P. plus one, to 68 per cent on L.P. plus two, 78 per cent on L.P. plus three, and 90 per cent on L.P. plus four. From that day on, only 90 per cent of the cases showed a mature corpus luteum, in spite of a good diphasic B.B.T. curve and a secretory endometrium.

These data suggest that the processes of ovulation and subsequent luteinization may have occurred in but 90 per cent of the patients with a diphasic B.B.T. curve and a secretory endometrium. In about 10 per cent luteinization may have occurred without prior external ovulation.

77. In Which Cases of Female and Male Sterility are Hormone Assays of Value?

L. I. Swaab, M.D.

From the Central Sterility-Clinic of the N.V.S.H., Amsterdam, Netherlands.

A survey is given of those endocrine glands and their hormones that influence the liberation of ova and spermatozoa. Hormone analyses, giving us information about the function of these glands, are critically discussed. Only in a small percentage of cases is sterility caused by hormonal disturbances. Consequently hormone assays have only a limited value in the study of sterility. In this paper, for the first time in the literature, strict rules are given for differentiating between those cases of male and female sterility in which hormone analysis might give us some basic information (and therefore should be carried out) and those in which they can readily be omitted. The FSH assay is the most important, as it informs us about the function of two glands: hypophysis and gonad. This assay is of value in a sterile patient with eunuchoidism, amenorrhea or with symptoms that might be due to Klinefelter's disease or to a climacterium virile. In these diseases the determination of the 17-ketosteroids and of estrone might also be indicated, but almost never in other cases of sterility. The value of the basal metabolism determination is briefly and critically reviewed.

78. The Simultaneous Administration of ACTH and Cortisone or Hydrocortisone.

Joseph W. Goldzieher, M.D., Earl Baker, M.D. (by invitation), and *W. B. Rawls, M.D.* (by invitation).

From the Research Department, St. Clare's Hospital, New York, N. Y.

The adrenal atrophy which accompanies prolonged therapy with cortisone and hydrocortisone poses an important clinical problem. For this reason a study was undertaken of the simultaneous administration of ACTH and adrenal steroid in rats. Adrenal weight and cholesterol concentration were used are criteria; the India-ink uptake of the reticuloendothelial system was also studied. Adrenals were examined at various intervals after hypophysectomy and compared to those of animals given (starting 1 week after hypophysectomy) 1 mg. of ACTH/day, 5 mg. of cortisone/day, 5 mg. of hydrocortisone/day, 1 mg. of ACTH and 5 mg. of cortisone/day, or 1 mg. of ACTH and 5 mg. of hydrocorti-

sone/day for fifteen days. Cholesterol was determined twenty-four hours after the last dose of ACTH. India-ink concentration was studied by a nephelometric method developed for this study.

The data will be interpreted in relation to the problem of maintenance of the adrenal cortex during steroid therapy and also with regard to direct *vs.* indirect effect of the steroid on the adrenal gland itself.

79. Stimulation by Exogenous ACTH Administration of an Adrenocortical Adenoma Producing Cushing's Syndrome.

F. O. Kolb, M.D., J. H. Bruce, M.D. (by invitation), *G. W. Liddle, M.D.* (by invitation), *G. M. Miller, M.D.* (by invitation) and *P. H. Forsham, M.D.*

From the Metabolic Unit, the Department of Medicine and the Department of Surgery (Urology), University of California School of Medicine, San Francisco, California.

A 41-year-old female with mild Cushing's syndrome showed consistently low 17-ketosteroid levels in the urine in the presence of elevated urinary 17-hydroxycorticoids. A standard eight-hour intravenous ACTH test showed an excessive rise of urinary 17-hydroxycorticoids. A right-sided adrenal tumor was demonstrated by retroperitoneal pneumography and confirmed pathologically. In the preoperative period an attempt was made to stimulate the atrophic adrenal on the side opposite to the tumor by administration of ACTH. This resulted in alarmingly high urinary corticoid levels, with accentuation of the clinical picture and a pre-psychotic state in the patient, necessitating temporary discontinuation of ACTH. In contrast, mild adrenal insufficiency developed after removal of the tumor, with a precipitous drop in steroid output. Repetition of an identical course of ACTH in the postoperative period produced only a slight rise in steroid output from the remaining atrophic adrenal. This demonstrates the successful stimulation of an adrenocortical adenoma producing Cushing's syndrome by ACTH and the absence of functional autonomy in this case. The response to ACTH stimulation does not, therefore, appear to differentiate between bilateral hyperplasia and an adenoma producing Cushing's syndrome.

80. Differential Intramuscular Inactivation of Highly Purified ACTH Preparations in Man.

P. H. Forsham, M.D., G. W. Liddle, M.D. (by invitation) and *D. Bleifer, M.D.* (by invitation).

From the Metabolic Unit and the Department of Medicine, University of California School of Medicine, San Francisco, California.

The employment of oxycellulose columns in the purification of ACTH has, during the past two years, resulted in the production of a number of ACTH preparations which, unlike the early crude preparations, do not induce refractoriness with prolonged use. Practically all of these purified preparations, however, are still subject to from 75 to 95 per cent inactivation when administered intramuscularly as compared to maximum effectiveness when administered intravenously under otherwise identical conditions. ACTH "A," a polypeptide prepared by Fisher *et al.*, was found to escape intramuscular inactivation in human subjects. However, ACTH "B," produced by partial hydrolysis of ACTH "A," was found to be nearly completely inactivated when administered intramuscularly, even though it retained full activity intravenously. Thus, a small change in molecular configuration, consisting in decreasing the chain length of an ACTH polypep-

tide, appears to make the preparation susceptible to intramuscular inactivation in man. The relative proportions of ACTH "A" and "B" in commercial ACTH preparations may conceivably determine their intramuscular effectiveness.

81. Hepatic Phospholipid Metabolism and the Adrenal.

Franz Halberg, M.D., Cyrus P. Barnum, Ph.D. and *Halvor Vermund, M.D.* (introduced by Maurice B. Visscher).

From the Department of Physiology and Physiological Chemistry, University of Minnesota, Minneapolis, Minn.

The inhibition of the 24-hour rhythms in rectal temperature and in tail-blood eosinophils, as well as a lower rectal temperature when compared with that of sham-operation controls, was noted for adrenalectomized CBC-mice, under standardized circumstances. These findings were interpreted as authentic evidence of cortical adrenal insufficiency. P^{32} was then injected intraperitoneally twice a day into independent subgroups from both groups of mice that had been operated on. Each mouse was killed two hours after injection of the tracer and its liver was removed for analysis. Relative specific activity of phospholipid phosphorus from liver microsomes (expressed as per cent of that of acid-soluble phosphorus) showed 24-hour periodicity in materials from sham-operation mice ($P<0.001$), but not in those from adrenalectomized mice ($P=0.58$). Moreover (regardless of time of sampling), the relative specific activity of phospholipid phosphorus was lower in materials from adrenalectomized mice ($P=0.002$). The significance of these data will be discussed.

82. Some Effects of Compound F and DCA on Organic and Inorganic Metabolism in the Adrenalectomized Rhesus Monkey.

Ernest Knobil, Ph.D. (by invitation), *Frederick G. Hofmann, Ph.D.* (by invitation) and *Roy O. Greep, Ph.D.*

From the Biological Research Laboratory, Harvard School of Dental Medicine, Boston, Mass.

Adrenalectomized monkeys were given compound F (1, 2 and 3 mg./Kg./day) and DCA (0.025, 0.05, 0.10, 0.20 and 2 mg./Kg./day) intramuscularly. The blood levels of sodium, potassium, chlorides, inorganic phosphorus, nonprotein nitrogen, amino-acid nitrogen and glucose, as well as the hematocrit and body weight, were measured at 3-day to 4-day intervals. Glucose tolerances were determined. Glycogen stores were estimated from blood sugar responses to epinephrine. Doses of 1 and 2 mg./Kg. of compound F only partially prevented weight loss and derangements in electrolyte concentrations, while maintaining the organic constituents near preoperative levels. However 3 mg./Kg. afforded complete replacement therapy in terms of the criteria used, except that glucose tolerance curves were slightly depressed. The blood sugar response to epinephrine was the same as that seen in intact monkeys. DCA, at all but the lowest dose, maintained the electrolytes at near-normal to normal concentrations. The other indices, likewise, did not differ significantly from control values, providing food was supplied without restriction. Twelve-hour fasting, however, severely lowered blood glucose concentrations, and convulsions, prostration and coma were of frequent occurrence, especially with the lower doses. Liver glycogen stores appeared to be normal in non-fasted DCA-treated animals, but were nearly obliterated following a twelve-hour fast.

83. Influence of Sex on the Excretion of an Acid Load by Adrenalectomized Rats.

Mary E. Dumm, Ph.D., Stephen H. Leslie, M.D. and *Bertram Laken, B.S.* (by invitation).

From the New York University College of Medicine, New York, N. Y.

Intact and adrenalectomized rats of both sexes were given 1.5 m-mols. of NH_4Cl (0.75 m-mols./ml.) per 100 grams of body weight by stomach tube after an eighteen-hour fast. The rats had access to 1 per cent NaCl until the start of the experiment. All intact rats and 80 per cent of the adrenalectomized rats survived the administration of NH_4Cl. Urine was collected for four hours and analyzed for $NH\pm^4$ and titratable acid. In agreement with the work of others (Pitts, R.: *Am. J. Med.* **9**: 356, 1950) the excretion of both NH^4 and titratable acid was depressed after adrenalectomy. However, it was also observed that after adrenalectomy the excretion of $NH\pm^4$ and titratable acid (in mEq./100 Gm. body weight) was higher in female than in male rats. The volume of urine excreted in the four hours after the administration of NH_4Cl was also higher in female than in male adrenalectomized rats; about 70 per cent of the ingested water was excreted by the females and about 50 per cent by the males. Intact rats receiving the same dose of NH_4Cl excreted 100 per cent of the ingested water in four hours. The response of intact rats to the administration of NH_4Cl was not consistently influenced by the sex of the animal.

84. Effect of Cold on Pituitary-Thyroid-Adrenal Relationships in the Guinea Pig.

C. E. Stevens, Ph.D. (by invitation), *S. A. D'Angelo, Ph.D., K. E. Paschkis, M.D., A. Cantarow, M.D.* and *F. William Sunderman, M.D.* (by invitation).

From Jefferson Medical College, Philadelphia, Pa.

The effects of cold on pituitary-target gland mechanisms were studied in young adult female guinea pigs chronically exposed to low environmental temperatures ($6+-2°$) for periods of six to eleven weeks. Control animals were maintained at room temperature ($23+-3°$ C.). Basal metabolic rates in cold-exposed animals were increased 25–35 per cent over normal. There was a marked increase in thyrotropic hormone content of the pituitary, with a concomitant rise in the blood TSH level. Histologic evidences of thyroid activation were seen but thyroid-body weight ratios, thyroidal radioiodine collections and serum protein-bound iodine concentrations were not significantly altered. Marked adrenal hypertrophy occurred in exposed animals. Ascorbic acid concentrations in the gland remained within normal limits. Excretion of corticosteroids (formaldehydogenic steroids) was increased 500–800 per cent.

The results indicate that in the guinea pig chronic exposure to cold activates both the pituitary-thyroid and pituitary-adrenal systems.

85. The Effects of ACTH and Adrenal Steroid Administration on Potassium Exchange in Human Erythrocytes.

David H. P. Streeten, M.R.C.P. (by invitation) and *Arthur K. Solomon, Ph.D.* (by invitation) (introduced by George W. Thorn).

From the Department of Medicine and the Biophysical Laboratory, Harvard Medical School, and the Medical Service, Peter Bent Brigham Hospital, Boston, Mass.

Unlike the loss of potassium induced by corticosteroids in other body cells, a *rise* occurs in the concentration of potassium within the erythrocytes following steroid

administration. Intravenous ACTH infusions cause a similar increase in erythrocyte potassium content, preceded in this case by a transient fall. These changes represent a true net flux of potassium, after correction for water shifts in the cells. Blood from normal subjects was incubated with K^{42} for four hours to determine separately the rates of influx and efflux of K. The rate of K influx was decreased 3–4, 8–10 and 22–30 hours after the start of ACTH infusions. The rate of efflux was also decreased. These effects were probably attributable to increased steroid blood levels, since they were reproduced by intravenous cortisone infusion. Furthermore, a dose-response relationship could be established when hydrocortisone was added to normal blood *in vitro*. Although gross influx and efflux changed *in vitro*, these changes were so correlated that no resultant change in net K flux could be demonstrated under our experimental conditions. These results suggest that the rise in the potassium content of erythrocytes *in vivo* may involve a mechanism more complicated than a simple, direct action of steroids on the erythrocytes.

86. Effect of ACTH and Epinephrine Upon Eosinophils, Urinary Sodium, Potassium and 17-Ketosteroids in Adrenalectomized Patients.

William McK. Jefferies, M.D., Richard P. Levy, M.D. (by invitation) and *Luther W. Kelly, M.D.* (by invitation).

From the School of Medicine, Western Reserve University, Cleveland, Ohio.

A single injection of ACTH in normal adults produces changes in circulating eosinophils and urinary excretion of sodium, potassium and 17-ketosteroids through stimulation of the adrenal cortex. Epinephrine, although producing similar changes in eosinophils, does not produce changes in urinary excretion characteristic of ACTH. This may be partially due to a separate effect of epinephrine upon renal excretion of Na and K. In order to obtain information as to whether either of these hormones increases peripheral utilization of adrenocortical hormone, the effect of single injections of each upon these metabolic effects was studied in 2 patients who had been bilaterally adrenalectomized as compared with 4 patients with Addison's disease. In some instances response was tested prior to cortisone therapy; in others, while on a constant dose of cortisone.

With ACTH there was no appreciable change in any of the effects studied. With epinephrine, eosinophils fell significantly in practically all tests, but there was no significant difference in urinary Na, K or 17-ketosteroids from ACTH or control tests. The results are interpreted as indicating that ACTH does not affect the rate of peripheral utilization of cortisone, at least as reflected by the changes studied. The fall in eosinophils with epinephrine could be interpreted as indicating increased peripheral utilization, but changes in urinary excretion of Na, K or 17-ketosteroids do not bear this out.

87. Modification of Steroid Excretion in Cushing's Syndrome by a High Fat Diet.

Edna H. Sobel, N.D., Leland C. Clark, Jr., Ph.D. (by invitation), *Solomon Papper, M.D.* (by invitation), *Sol Sherry, M.D.* (by invitation) and *Gwen Prior* (by invitation).

From the Departments of Pediatrics and Medicine, University of Cincinnati, The Fels Research Institute, Yellow Springs, and the May Institute for Medical Research, Cincinnati, Ohio.

In 1952 Kinsell observed (*Ann. Int. Med.* **37**: 921) that 17-ketosteroid excretion did not rise as expected in ACTH-treated individuals fed a high fat diet. This report concerns the 17-ketosteroid and formaldehydogenic corticosteroid excretion of a 13-year-old boy

with Cushing's syndrome due to bilateral adrenal cortical hyperactivity, on a normal diet and on an isocaloric high-fat, low-carbohydrate regimen. There was a decrease in both 17-ketosteroid and corticosteroid excretion during the first four days of the high fat regimen ($p=0.03$). In the succeeding three days of the modified diet, both measurements showed a gradual increase to higher than control levels. When the diet was returned to normal the steroid excretion did not differ significantly from that of the first control period. There was no ketosis, no consistent change in urine volume, or any change in weight, blood pressure, clinical status or salivary electrolytes.

	Days	17-Ketosteroids, average mg./day	Corticosteroids average mg./day
Control	8	11.68	0.479
High fat	4	10.32	0.328
	3	15.53	0.567
Control	5	12.07	0.463

88. Effects of Injury and Dietary Factors on Adrenal Weight and Cholesterol Concentration.

Elaine P. Ralli, M.D., Mary E. Dumm, Ph.D. and *Herbert Gershberg, M.D.*

From New York University College of Medicine, New York, N. Y.

The effect of injury on adrenal weight and cholesterol concentration was determined in male rats on three diets: 1) a diet adequate in all nutritional factors for rats, 2) a diet deficient in pantothenic acid, and 3) a diet containing excessive amounts of pantothenate. After thirty days on the diet, unilateral adrenalectomy was performed. In the injury experiments, in addition to unilateral adrenalectomy, an amount of skin equivalent to 4 per cent of the body weight was removed. In each case the weight and cholesterol concentration of the adrenal were determined and the remaining adrenal removed and analyzed after periods up to seven days.

Unilateral adrenalectomy was associated with a decrease in cholesterol at six hours in all diet groups. At twenty-four hours the cholesterol concentration approximated its original value in the control and high-pantothenate rats, but had further decreased in the deficient rats. At three, five and seven days the cholesterol concentration approximated the preoperative levels in the control and high-pantothenate rats.

In contrast, after wounding, the adrenal cholesterol did not return to the preoperative level until five to seven days. In the high-pantothenate wounded rats the adrenal cholesterol fell less at six and twenty-four hours than in the controls.

Following the unilateral adrenalectomy alone, the weight of the remaining adrenal increased moderately, but in the wounded animals the remaining adrenal gland approximately doubled its weight by five to seven days after injury.

89. The Metabolism of β-Estradiol* in Man.

Benjamin F. Stimmel, Ph.D. and *James A. May, M.D.* (by invitation).

From the Rees-Stealy Clinic Research Foundation and the Department of Urology of the Rees-Stealy Clinic, San Diego, California.

As judged by the urinary estrogen excretion pattern determined spectrophotometrically, following treatment with a small oral dose (10.0 mg.) of β-estradiol, man is in-

capable of converting this C-17 epimer of α-estradiol, to estrone, α-estradiol and estriol. Unchanged β-estradiol is the only recognizable urinary estrogen excretion product observed following such treatment. The urinary β-estradiol is excreted in a form which is β-glucuronidase (bacterial) hydrolyzable. Recovery in the urine did not exceed 5 per cent in any of the cases studied. These studies would suggest that the enzyme system involved in the conversion of α-estradiol to estrone is highly specific, since man readily converts α-estradiol to estrone, even in the presence of extensive liver damage (Stealy, C. L. and Stimmel, B. F.: Further studies on the metabolism of therapeutic doses of natural estrogens in human subjects, *J. Clin. Endocrinol.* **8**: 67, 1948).

* Old nomenclature. This compound is now designated as estradiol-17α.

90. Quantitative Patterns of Urinary Corticosteroids.

Hildegard Wilson, Ph.D., Richard Fairbanks (by invitation) and *Dominick Scialabba, A.B.* (by invitation).

From the Study Group of Rheumatic Diseases, and the Department of Chemistry, New York University College of Medicine, New York, N. Y.

Urinary extracts made before and after hydrolysis with β-glucuronidase have yielded corticosteroids of various mobilities on benzene-formamide papergrams. The slow, medium, and fast moving compounds have been separated, and further divided according to triphenyltetrazolium tests. Quantitative assays on eluates from 1 case of Cushing's syndrome and 1 of pregnancy, compared with those of normal males, indicate that variations in distribution of corticosteroid metabolites accompany differences in adrenal cortical function. In Cushing's syndrome, formaldehydogenic assays on slow compounds were 5 times normal; faster compounds were 2 to 4 times normal. Porter-Silber assays also indicated a preferentially elevated excretion of slow-moving $C_{21}O_5$17-hydroxy α-ketols. Abnormal 17-hydroxy α-ketols were suggested by positive results with Porter-Silber assays on faster fractions which showed negative results in normal males. In pregnancy, formaldehydogenic assays were about twice normal in all regions, showing the expected increase in total corticosteroid. However, the corticosteroid mixture contributing these titers appeared different from the others, since the slow material gave low Porter-Silber titers, whereas medium compounds were 4 times normal.

91. Studies on the Colorimetric Estimation of Estrogens.

Desmond Beall, Ph.D. (by invitation) and *Gordon A. Grant, Ph.D.*

From the Research and Biological Laboratories, Ayerst, McKenna & Harrison Ltd., Montreal, Canada.

Since the publication in 1934 of the Marrian-Kober reaction for estrogens, a number of modifications have been proposed giving more specific colors for the individual members of this group. The present communication is a report of some results obtained in our laboratories on this problem. Based on Haenni's work in 1950 that the color development of the Kober reaction is affected by minute quantities of iron, the effects of micro quantities of various metals have been studied. Data relating to the inclusion of cobalt (5γ/ml.), which increases the absorption at 525 mμ and decreases it at 420 mμ, will be presented. Variations in color intensity resulting from the quality of the H_2SO_4 employed are also minimized in the new reagent. In 1950 Banes obtained a blue color with equilin, using a modified Haenni iron-Kober reagent. Replacement of the iron by cobalt and inclusion of HCl yielded a quantitative reagent giving a blue color with equilin

on heating, and a yellow color with α- or β-dihydroequilin at room temperature. The other nonketones do not react under similar conditions. By employing naphthanil Diazo Blue B, a coupling reaction specific for β-naphthol derivatives has been devised, permitting the estimation of equilenin and α- and β-dihydroequilenin in estrogen mixtures.

92. Bioassay of Adrenal Steroids in Blood.

Eugenia Rosemberg, M.D. (by invitation), *Jerome Cornfield, B.S.* (by invitation), *Robert Bates, Ph.D.*, and *Evelyn Anderson M.D.*

From the National Institutes of Health, Bethesda, Md.

A bioassay technique (previously described) based on the eosinophil response to graded doses of cortisone of adrenalectomized mice (C-57 brown) was applied to a study of blood steroids. Fifty per cent propylene glycol solutions of a defatted, unfractionated ethyl acetate:ethyl ether (2:1) extract of heparinized plasma or whole blood were injected into the test animals.

Forty micrograms of cortisone were added to 30 ml. of peripheral blood of a dog, and then extracted; 36.4 micrograms (40.8-31.2) (68 per cent confidence limits) of cortisone equivalents were found. A parallel sample of 30 ml. of peripheral blood yielded an estimate of endogenous cortisone equivalents of 2.2 micrograms (5.4-0.6). The recovery thus appears substantially complete within the limits of the error of the assay.

The adrenal steroids of dog's blood were measured. The output of cortisone equivalents in the left adrenal vein was found to be 93 micrograms (118-72) per 100 ml. of plasma at the beginning of collection and 165 micrograms (211-120) per 100 ml. two and a half hours later. In the cat the output by both adrenals was 90 micrograms (92-87) per 100 ml. of plasma. After the administration of ACTH to the dog the cortisone concentrations were: peripheral vein, 44 micrograms (84-24) per 100 ml. of plasma; vena cava, 44 micrograms (57.2-31.0) per 100 ml.; and adrenal vein, 223 micrograms (296-142) per 100 ml. (corresponding to an output of 3.1 micrograms (4.1-2.0) per minute).

The estimated concentration in peripheral blood in a normal human subject was 11.2 micrograms (15.2-8.6) per 100 ml. of plasma. In a patient with Cushing's disease the concentration was 51 micrograms (73-29) per 100 ml.

93. Steroid Studies: I. Metabolic Effects of Androstanol-17β-one-3 in Aged People.

Sidney Pearson, Ph.D., Jonas Weissberg, M.D. and *Thomas H. McGavack, M.D.*

From the New York Medical College, Metropolitan Hospital, Research Unit, Welfare Island, New York, N. Y.

Six patients who varied in age from 55 to 75 years were given a constant diet for twenty days. Beginning on the fifth day of this diet, 5 subjects were given daily, for twelve days, 25, 50 or 100 mg. of androstanol-17β,one-3 (A); the sixth subject (the control) received a placebo. During the recovery period, the seventeenth through the twentieth days, no A was given. Every two days, two-day aliquots of food were analyzed for sodium, potassium, chloride and nitrogen. Forty-eight hour urinary specimens were analyzed for sodium, potassium, chloride, nitrogen, 17-ketosteroids and creatinine. Determinations of sodium, potassium, chloride, total serum protein, albumin, globulin and total cholesterol in blood were made on the fourth, sixteenth, and twentieth days, and showed no variations attributable to the steroid. Daily doses of 25, 50 or 100 mg.

of A produced an increase in nitrogen retention accompanied by an increase in weight. Administration of 25 mg. of A did not affect the urinary excretion of 17-ketosteroids, whereas administration of 50 and 100 mg. was associated with an increase. In all cases androstanol-17β,one-3 produced a marked feeling of well being.

94. Steroid Studies: II. Metabolism of Androstanol-17β,3-one.

Sidney Pearson, Ph.D. and *Thomas H. McGavack, M.D.*

From the New York Medical College, Metropolitan Hospital, Research Unit, Welfare Island, New York, N. Y.

A 43-year-old agonadal obese male was given a constant diet for twenty days. On the fifth day of the diet and for the following eleven days, the subject received daily 50 mg. of androstanolone. The results of urine and food analyses showed that androstanolone produced a decrease in the excretion of urinary nitrogen and an increase in the output of urinary 17-ketosteroids. The urinary excretion of sodium, potassium, and chloride was not affected. The total urinary 17-ketosteroids excreted for three days before the beginning of androstanolone administration were collected and an aliquot was chromatographed over alumina. Forty-four fractions were obtained and assayed for 17-ketosteroids. Similarly, the total urinary 17-ketosteroids excreted over a period of six days, during which time the subject received daily 50 mg. of androstanolone, were combined and chromatographed. The results show that, at the dosage level studied: 1) only a small fraction, if any, of androstanol is excreted unchanged; 2) androstanol suppresses the excretion of some ketosteroids; and 3) androstanolone alters the pattern of ketosteroid excretion.

95. A Micro Spectrophotometric Technique, of Use in the Identification of C-19 Steroids.

Lytt I. Gardner, M.D.

From the Department of Pediatrics, State University of New York Medical School at Syracuse, N. Y.

Due to the increasing need for auxiliary means of identifying small amounts of steroids obtained by chromatography, a method has been devised utilizing the absorption maxima of C-19 steroids between 210 mμ and 700 mμ in sulfuric acid at three standardized conditions of acid concentration and temperature. There are specific shifts of absorption maxima under these conditions, thus multiplying the usefulness of the data for characterization purposes. Since the same sample may be used to obtain all three sets of maxima, it is possible to work with as little as 1 μg. of steroid. The following steroids have been so characterized: androstanedione-3-17; etiocholan-3α-11β-diol-17-one; Δ^5-androstenediol-3β-17β; epiandrosterone (androstane-3β-ol-17-one); androsterone; dehydroepiandrosterone; testosterone; and androstanediol. Plasma from normal adults and children with congenital adrenal hyperplasia was extracted with ether, partitioned between hexane and 70 per cent ethanol and chromatographed on silica gel-magnesia columns. Data obtained by the application of the foregoing technique to these fractions are discussed.

96. Quantitative Fluorometry of Estrogen Mixtures.

Joseph W. Goldzieher, M.D.

From the Research Department, St. Clare's Hospital, New York, N. Y.

The increasing use of fluorometry for the measurement of estrogens emphasizes the need for evaluation of various methods and standardization of the optimum technique. Factors involved in the reproducible development of maximum fluorescence, such as the preparation of reagents and purification of sulfuric acid, and the use of 405 or 436 mμ radiation will be illustrated. On the basis of fluorescence spectra, an optical method for the measurement of estrone and estradiol-17β in binary mixtures has been developed, thus avoiding chemical or physical separations of mixtures otherwise sufficiently pure. With selected filters, measurement at 470 mμ gives equal fluorescence energies for estrone and estradiol, whereas at 400 mμ estrone is 1/10 as fluorescent as estradiol. Other filters can measure the two compounds at an intensity ratio the same as the ratio of their biologic activities. The basic data for the measurement of mixtures and the criteria for filter selection will be outlined.

97. An Effect *In Vitro* of Cortisone on Intracellular Potassium and Sodium Concentrations of Rabbit Leucocytes.

D. Laurence Wilson, M.D.

From Queen's University, Kingston, Ontario.

Suspensions of leucocytes were obtained by aspiration of the peritoneal cavities of rabbits, eighteen hours after intraperitoneal injection of sterile 0.9 per cent NaCl solution. For each experiment, the pooled suspension from 2 or more animals was divided into two equal portions. To one was added an amount of a 250-mg./100 cc. cortisone solution in 95 per cent ethanol, to produce a final concentration of 10–20 mg./liter; to the other portion, an equal volume of 95 per cent ethanol. Both were gently aerated in a water bath at 37.5° C. for one hour, and then centrifuged for forty-five minutes at 2000 r.p.m. Cells and supernatant liquid were analysed for sodium and potassium content by flame photometry after dry ashing.

Cell potassium concentrations normally ranged from 75 to 143 mEq./Kg. of cell water; cell sodium concentrations bore a reciprocal relation to cell potassium, ranging from 57 to 109 mEq./Kg. of cell water. In 11 experiments, the addition of cortisone (20 mg./liter) induced an average fall in cell potassium concentration of 4.6 mEq./Kg. of water (0 to 8.7) without change in cell water—statistically significant beyond the 1 per cent level; reciprocal increases in cell sodium occurred. Preliminary experiments show a similar effect of desoxycorticosterone glycoside.

98. Differences in the Effect of Cortisone, Compound F and ACTH on the Thyroid, as Revealed by Microhistometry.

Boris Catz, M.D., M.Sc., Ihsan El-Rawi, M.Sc. (by invitation), *Ernest Geiger, M.D., Ph.D.* (by invitation) and *Paul Starr, M.D.*

From the Departments of Medicine, Biochemistry, Nutrition, and Pharmacology, University of Southern California School of Medicine, Los Angeles, California.

Rats kept at room temperature (24–26° C.) showed a statistically significant increase in thyroid cell height when receiving 5 mg. of cortisone acetate intraperitoneally per day for fifteen days. ACTH in doses of 2 mg. per day for fifteen days did not produce this increase. The normal values of 24-hour collection of radioactive iodine by the thyroid gland were essentially not changed by the hormones used. In preliminary experiments, hydrocorticosterone acetate, when given in amounts of 5 mg. intraperitoneally per day

for fifteen days, did not show this increase in cell height observed with cortisone. The finding is being further investigated.

When the animals were fasted for twenty-four hours immediately after receiving the tracer injection a twofold increase in the 24-hour collection of radioactive iodine was observed in both groups of treated and control animals.

To establish whether the increased cell height of the thyroid gland observed in animals exposed to cold is affected by the administration of the hormones, animals that were exposed to cold (3° C.) for eight days received during that period, daily intraperitoneal injections of 5 mg. of cortisone acetate, or 2 mg. of ACTH.

It was observed that the cortisone treatment did not change the increased thyroid cell height resulting from cold exposure. When ACTH was administered the hyperplasia produced by cold could not be observed, giving values similar to those of normal room temperature animals.

All the animals used were young males of the Wistar strain and were fed Purina Chow containing 100 gamma of iodine per 100 grams (average).

The following table summarizes the results obtained in a total of 60 animals.

	15 days, 24–26° C.			8 days	3° C.
	MACH*	24-Hour I^{131} uptake		MACH	I^{131} uptake
		Fed	Fasted		
Control	11.9	15.6	31.0	14.0	13.3
Cortisone	13.7	12.6	32.8	14.3	14.5
ACTH	10.9	12.9	28.0	11.6	15.2
Compound F	11.0	—	40.9	—	—

* Mean acinar cell height (micra).

The site and mode of action of the corticoids with regard to the thyroid will be discussed and the controversial literature analyzed.

99. Critical Evaluation of Methimazole (1-Methyl-2-Mercaptoimidazole) in the Treatment of Hyperthyroidism.

Jacqueline Chevelley, M.D., Samuel Kenigsberg, M.D., Adolph Gerl, M.D. and *Thomas H. McGavack, M.D.*

From the New York Medical College, Metropolitan Hospital, Research Unit, Welfare Island, New York, N. Y.

Approximately 150 patients with some form of hyperthyroidism have been treated to date with Methimazole, either in preparation for surgery or as part of a program of long-term medical care. Approximately 80 per cent of these were women; 30 per cent had a nodular toxic goiter. Approximately 90 per cent of all patients were controlled with Methimazole, if the dosages were sufficiently large. Nearly half of the patients were prepared for surgery and in this group Methimazole never failed to evoke a satisfactory response. Of the medically followed cases upon whom satisfactory study could be completed, approximately one third showed complete recovery which was maintained for more than twelve weeks after withdrawal of the drug. Based on this study, a comparison

of Methimazole with thiouracil, 5-iodothiouracil, methylthiouracil and propylthiouracil reveals the following: Methimazole acts the most quickly of these compounds; its beneficial effects are attained by doses varying from one twenty-fifth to one tenth of those necessary for control with thiouracil, propylthiouracil or methylthiouracil. Satisfactory maintenance doses are more difficult to achieve with Methimazole than with methylthiouracil or thiouracil, as there is a tendency for previously satisfactory dosages to become ineffective or overly active without warning. Methimazole was by far the most convenient of these compounds for preparing patients for surgery, as its action is readily manifest within three to five days, with control usually complete in not more than twenty-one days if dosages have been sufficient. It is our impression that its action is associated with a less vascular gland than is seen with the thiouracils. It has the added advantage of being relatively free of toxic symptoms. Toxic effects have occurred in from 2 to 3 per cent of our cases treated with Methimazole, and include granulocytopenia (no instances of complete agranulocytosis), generalized and localized rashes, edema and itching.

100. Effects of Altered Thyroid Function Upon the Metabolism of Endogenously Labeled Hormone.

A. Albert, M.D.

From Mayo Clinic, Rochester, Minnesota.

Thiouracil caused an intense loss of thyroidal I^{131} and a symmetrical increase in I^{131} of the rat gastrointestinal tract and carcass. Thyrotropin induced similar but less intense loss of thyroidal I^{131} and a symmetrical gain in I^{131} of the carcass and gastrointestinal tract. Hypophysectomy, or therapy with thyroglobulin, produced a marked increase in thyroidal I^{131} and a marked and asymmetrical decrease in the extrathyroidal I^{131} with a greater decrease occurring in the gastrointestinal tract than in the carcass I^{131}. There is a wide range over which the excretion of I^{131} from the body is proportional and in equilibrium with the secretion of I^{131} from the thyroid. When liberation of thyroidal I^{131} is inhibited by thyroglobulin, the excretion of I^{131} in the feces and urine is correspondingly inhibited. However, when liberation of thyroidal I^{131} is accelerated by thouracil, the fecal and urinary excretion of I^{131} is also accelerated but does not keep pace with the liberation of I^{131} from the thyroid. The I^{131} excreted in the urine and feces, or present throughout the tissues of the body is in equilibrium with the I^{131} of the thyroid, but not with the total I^{127} present in the rat.

101. Comparative Effects of Thyroxine and Triiodothyronine in Thyroidectomized Rats.

Arthur E. Heming, Ph.D. and *Dorsey E. Holtkamp, Ph.D.*

From the Research and Development Division, Smith, Kline and French Laboratories, Philadelphia, Pa.

In groups of 6 rats each, thyroidectomized animals were treated in a molar ratio of 3.5 to 1 with; first, L-thyroxine sodium pentahydrate, and later, after an intervening control period, with 3:5:3′-triiodo-L-thyronine. The daily subcutaneous administration of 6, 12, 24, and 60 γ/Kg./day of thyroxine for seventeen days, elevated the oxygen consumption to about the same respective levels as similarly administered doses of 1.25, 2.5, 5.0, and 12.5 γ/Kg./day of triiodothyronine. However, triiodothyronine at 12.5 γ increased oxygen consumption more than did thyroxine at 60 γ.

An acute, subcutaneous, single dose of 312 γ/Kg. of triiodothyronine produced a rise

in oxygen consumption qualitatively similar to that produced by a single dose of 1,500 γ/Kg. of thyroxine. There was no detectable elevation in oxygen consumption when measured four hours after the time of injection. After eight hours, however, each drug had produced a significant rise. Furthermore, the period of greatest response to each agent extended from about twenty-four to eighty hours after the time of injection. The "peak response" occurred sometime during this interval. In this experiment, thyroxine induced a higher response than triiodothyronine.

Based on oxygen consumption studies in thyroidectomized rats, therefore, the molar activity ratio of triiodothyronine to thyroxine is between 3:1 and 4:1.

102. Etiologic Factors in Sporadic Cretinism: An Analysis of 90 Cases.

Barton Childs, M.D. (by invitation) and *Lytt I. Gardner, M.D.*

From the Harriet Lane Home, Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University, Baltimore, Md.

Clinical data on 90 congenital, sporadic, nongoitrous cretins have been analyzed by the genetic statistical methods devised by Penrose and by Haldane. The Wilkins-Fleischmann series of cretins comprised most of the cases which were studied (*cf. J.A.M.A.* **116:** 2459, 1941). Many of the data were obtained directly from the parents or by questionnaire. Comparison was made with a normal control group of 78 families in the same geographic area, from whom comparable data were elicited. The findings were as follows: 1) There was a significant familial incidence of thyroid disease, not necessarily cretinism. 2) There was no unusual incidence of consanguinity, and only 2 cretins had cretinous siblings. The latter findings argue strongly against the conditions being a simple, Mendelian recessive trait. 3) There were but 2 Negro cretins, although more than two thirds of the cases came from Maryland and the District of Columbia. 4) There was a tendency for the cretins to be the offspring of older, multiparous mothers. 5) The cretins were significantly heavier at birth than the normal babies, and a frequency distribution curve of the birth weights showed a bimodality which suggested two groups of cretins—one with relatively normal weights, the other with much greater weights at birth. Comparison with maternal age indicated that the heavier babies were the product of older mothers. 6) There was no seasonal incidence of the disease.

103. Changes in Urinary Nitrogen Excretion During Stressful Life Experiences and Their Relation to Thyroid Function.

Basil S. Hetzel, M.D., William W. Schottstaedt, M.D., Lawrence E. Hinkle, Jr., M.D. and *Harold G. Wolff, M.D.* (introduced by Willard O. Thompson, M.D.)

From the Departments of Medicine and Psychiatry, New York Hospital—Cornell Medical Center, New York, N. Y.

Previous work has demonstrated a rise in PBI in association with certain stressful life experiences. Some of the metabolic accompaniments of this phenomenon have been investigated.

In fasting subjects the urinary nitrogen excretion has been measured by a modified micro-Kjeldahl procedure and no significant change has been observed when the PBI level remains steady. During stressful life experiences an increase of up to 80 per cent in nitrogen excretion has been found to occur, in association with a rise in the PBI. Simultaneously, diuresis of water and potassium was also observed. The increase in nitrogen excretion has been found to account for most of the increase in potassium excretion.

Under the same conditions, hypothyroid subjects maintained on desiccated thyroid showed no significant increase in nitrogen excretion and no rise in PBI, though a diuresis was observed.

It is provisionally concluded that, under the conditions of these experiments, there is an increase in nitrogen excretion from increased catabolism in association with an increase in output of the hormone from the thyroid.

104. Effects of *p*-Aminosalicylic Acid on Thyroid and Adrenal Function.

John R. Hogness, M.D. (by invitation), *Thomas Wong, M.D.* (by invitation) and *Robert H. Williams, M.D.*

From the Department of Medicine, School of Medicine, University of Washington, Seattle, Wash.

Recent clinical observations have indicated that goiter and, rarely, transient myxedema may occur in patients treated with *p*-aminosalicylates over prolonged periods. However, despite conflicting reports, it is probable that short-term administration of PAS has little or no effect on the pituitary-adrenal axis. An experiment was designed to study the effects of prolonged administration of this compound on rat thyroid and adrenal function. Rats were fed 1 per cent powdered PAS in the diet for two weeks. At the end of this period, thyroid weight and radioiodine uptake were determined. At the same time, measurements of adrenal weight, adrenal cholesterol and ascorbic acid concentrations, circulating eosinophils, liver glycogen and thymus weight were made. The thyroid glands of animals receiving 1 per cent *p*-aminosalicylic acid in the diet weighed twice as much as those of controls, and the ability of the glands to concentrate radioiodine was reduced to 20 per cent of normal. Whereas the inhibition of I^{131} uptake was noticed in hypophysectomized animals, thyromegaly was not. This indicates that PAS acts as a thyroid-blocking agent, in a manner similar to thiouracil. Our studies showed, however, that propylthiouracil in concentrations of 0.2 per cent in the diet, is more effective as a blocking agent than is PAS in 1 per cent concentration. Although an increase in liver glycogen concentration was observed in PAS-treated animals, there was no change in any of the other adrenal properties studied. The mechanism of the rise in liver glycogen is being investigated.

105. Cabbage and Turnips as a Cause of Endemic Goiter.

Isidor Greenwald.

From the Department of Chemistry, New York University College of Medicine, New York, N. Y.

Suk (*Anthropologie* **9**: 1–6, 1931) seems to have been the first to suggest that an excessive consumption of cabbage might be the cause of endemic goiter. He found that the poorer inhabitants of Carpatho-Ruthenia ate about 500 grams of cabbage per day throughout the winter. This amount seemed extraordinary to Suk, but it is not so unusual as he supposed. Instances of an equally great consumption in healthy families can be found elsewhere and the statistics for Germany and even for the United States indicate that there must be many families in which the intake is nearly or quite as great. The use of turnips for the feeding of cattle in winter is widespread and, in England, quite general. The supposed production of goiter in Belgian monks by cabbage and rape-seed or by rutabagas and tulip bulbs could not be verified. Moreover, cabbage and turnips inhibit the uptake of iodine by the thyroid, whereas the usual goitrous gland takes up iodine at least as readily as does a normal gland.

106. The Difficulties in the Clinical Management of Hermaphrodism.

Henry H. Turner, M.D., R. Palmer Howard, M.D., F. M. Lingenfelter, M.D. (by invitation) and *James P. Dewar, M.D.* (by invitation).

From the University of Oklahoma School of Medicine and Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma.

True lateral hermaphrodism is very rare. As a child the patient was considered a boy, with penile chordee, perineal hypospadias and right cryptorchidism. Pubic hair was noted at the age of 13 but male secondary characteristics failed to appear. Therefore, plastic procedures to bring the urethral orifice to the normal male position were started at the age of 15. During the next two years the development of large breasts and the finding of an ovo-testis upon left scrotal gonad biopsy raised doubts as to the sex. When peritoneoscopy failed to reveal female internal viscera, bilateral mastectomy was performed. At the age of 18 regular monthly urethral bleeding again raised the question, and eventually a vaginal cavity with a small sinus into the membranous urethra was detected with the endoscope. At laparotomy a uterus, a normal right tube and ovary, and an abnormal duct to the left scrotal gland were found. Because the patient was socially adjusted as a male, and because of the previous plastic procedures, masculinization was completed. Eight months later the patient was without complaints and was receiving endocrine therapy.

It is concluded that in cases with marked genital deformities every effort should be made in early childhood to determine the true nature of the gonads before the external structures are altered and before the sexual adjustment of the child is firmly established.

107. The Effectiveness of Oral Cortisone in Sjoren's Syndrome.

Manuel M. Villaverde, M.D.

Hospital Ntra. Sra. De Las Mercedes, Habana, Cuba.

The case is that of a white male, 47 years of age. The lack of saliva, together with the lack of tears, a great reduction of sweating, and other systemic symptoms such as dyspepsia, rheumatic pains, and neurotic troubles led us to the diagnosis of Sjogren's syndrome. Most of the regular symptoms of the complex were present, with the exception of lymphocytosis; instead, we found lymphopenia.

Three grams of cortisone were given by the oral route in one month. It proved curative in the sense that it restored the normal flow of tears and saliva; it also improved the eye condition (keratoconjunctivitis sicca), the rheumatic pains, and most of the symptoms of the complex; but reactions were easily induced. Three months after withdrawal of the hormone the patient was found in good condition.

108. Primordial Dwarfism with Pancytopenic Hypersplenism in 2 Siblings.

Theodore C. Panos, M.D.

From the University of Texas Medical Branch, Galveston, Texas.

Two strikingly dwarfed male, successive siblings were observed for four years and detailed autopsy studies were obtained in one. In each instance, normal delivery followed full-term, uncomplicated gestation, and birth weight was approximately 1,500 grams. Changes in body proportions, dentition and bone age were commensurate with chronologic age, although each weighed only 5 kilograms and measured approximately 75 centimeters in length at the ages of 5 and 6 years, respectively. Both were definitely

mentally retarded. Consanguinity between parents was denied; the mother had given birth to 2 normal half-siblings and 1 normal older female full sibling.

Serial skull roentgenograms revealed a normal sella turcica. There was no clinical or laboratory evidence of failure of elaboration of adrenocorticotropin or thyrotropin, or any of the "inborn errors of metabolism." Both were cryptorchid. Attempts to stimulate growth by use, alone or in combination, of thyroid substance, growth hormone, testosterone and vitamin B_{12} were completely unsuccessful. Severe pancytopenic hypersplenism constituted the critical problem in management. Splenectomy in 1 patient resulted in moderate improvement in maintenance of peripheral erythrocyte and hemoglobin levels but failed to alter significantly the granulocytopenia or thrombocytopenia. Splenectomy was performed in the other patient but death ensued from postoperative hemorrhage. Autopsy revealed a pituitary of normal size and cytology.

109. The Hypothalamus in Cushing's Syndrome Due to Bilateral Adrenal Hyperplasia.

Thomas H. Maren, M.D. (introduced by E. B. Astwood, M.D.).

From the Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore, Maryland, and the Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut.

It has been claimed (Heinbecker, P.: *Medicine* **23**: 225, 1944) that in cases of Cushing's syndrome with bilateral adrenal hyperplasia, there is marked deficiency of hypothalamic cells, particularly those of the paraventricular nuclei. The suggestion was that this deficit represents the pathologic basis for the endocrine disorders in this disease.

In view of the great interest in relations between the hypothalamus and the anterior pituitary-adrenocortical system, the foregoing concept was re-examined, using quantitative methods of cell counting.

The diencephalon was obtained from formalin-fixed autopsy material of 2 young women who had been studied during life at the Johns Hopkins Hospital and in whom the diagnosis of Cushing's syndrome was unequivocal. In both cases the adrenals were symmetrically enlarged, without tumor, and there was thickening of the *zona reticularis*. One patient had a minute basophil adenoma of the pituitary; Crooke's changes were clear in one case and probable in the other. A control brain was used from a patient in the same age and sex group, without endocrine or neurologic disease. The hypothalami of these 3 patients were cut in 15–20μ sections from paraffin and stained with toluidine blue or gallocyanin. Every twentieth to thirtieth section was examined. Cell counts of the large-cell paraventricular and supraoptic nuclei were made, and other hypothalamic groups were also studied.

No significant differences were found between control material and that from Cushing's syndrome. Morphologically, the hypothalamus appeared normal; Heinbecker's observation could not be confirmed. The present findings throw no light on the nature of hypothalamo-adenohypophysial relations, except to suggest that the hypothalamus may be histologically normal in the presence of endocrinopathy. This has also been noted by the writer in the case of the genetically dwarfed mouse—in the virtual absence of the anterior pituitary, the hypothalamic nuclei appear normal.

110. The Incidence of Endocrine Dysfunction Following Postpartum Hemorrhage and/or Shock.

Norman G. Schneeberg, M.D., William H. Perloff, M.D. and *S. Leon Israel, M.D.* (by invitation).

From the Endocrine Clinics, Philadelphia General Hospital, the Endocrine Clinic and Research Department, Southern Division, Albert Einstein Medical Center, and the Endocrine Laboratory, Temple University School of Medicine, Philadelphia, Pa.

Studies designed to evaluate anterior pituitary, thyroid, adrenocortical and ovarian function were performed on 17 survivors of postpartum hemorrhage and/or shock, to determine the incidence of adenohypophyseal insufficiency (Sheehan's syndrome) in apparently healthy women, and to attempt to detect subclinical Sheehan's syndrome. Abnormalities were found in 3 instances: 1) panhypopituitarism, 2) adrenocortical hypofunction plus ovarian failure, and 3) ovarian failure. In the latter 2 instances the amount of blood loss and the degree of circulatory collapse were uncertain but were undoubtedly mild. There was no correlation between the severity of postpartum complications and endocrinopathy. Three subjects who required hysterectomy escaped detectable pituitary damage. Fourteen women were apparently normal, though 3 showed certain clinical and laboratory stigmata suggesting possible endocrine dysfunction. Three subjects had subsequent pregnancies. Two, excluding the hysterectomized women, were amenorrheic. Two failed to lactate and 2 showed reduced lactation in the puerperium. The findings indicate that unsuspected endocrine dysfunction following postpartum hemorrhage and/or shock can be detected by appropriate diagnostic measures. The condition is not as frequent as one might anticipate, particularly in patients who have experienced profound prolonged shock.

THE SIXTH ANNUAL POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF MINNESOTA AT THE CENTER FOR CONTINUATION STUDY OF THE UNIVERSITY OF MINNESOTA MEDICAL SCHOOL, MINNEAPOLIS, MINNESOTA

September 28–October 3, 1953

The faculty will consist of fifteen clinicians and investigators eminently qualified in the fields of endocrinology and metabolism. The program will consist of lectures, demonstrations of laboratory tests, presentation of cases, and question and answer panel discussions.

The forty-five lectures to be presented will cover most of the phases of endocrinology and certain metabolic diseases. The course is designed to give a broad basis of understanding of diagnosis and therapy in the field of endocrinology and metabolism for the man in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of the lectures will be available to the registrants at the time of the Assembly.

Formal announcements and application forms will be available shortly. *For further information concerning the program, write to the Director, Center for Continuation Study, University of Minnesota, Minneapolis 14, Minnesota.* Arrangements for housing accommodations will be made through the Center for Continuation Study.

FACULTY

Dr. Alexander Albert, Consultant and Head, Endocrine Laboratory, and Assistant Professor of Physiology, Mayo Clinic, Rochester, Minnesota.

Dr. Earl T. Engle, Professor of Anatomy, Columbia University, College of Physicians and Surgeons, New York, N. Y.

Dr. Gerald T. Evans, Professor of Medicine and Director of Laboratories, University of Minnesota Medical School, Minneapolis, Minnesota.

Dr. Edmund B. Flink, Associate Professor of Medicine, University of Minnesota Medical School, Minneapolis, Minnesota.

Dr. Robert B. Greenblatt, Professor of Endocrinology, Medical College of Georgia, Augusta, Georgia.

Dr. Lewis M. Hurxthal, Head, Department of Internal Medicine, Lahey Clinic, Boston, Massachusetts.

Dr. Joseph W. Jailer, Associate in Medicine, College of Physicians and Surgeons, Columbia University, New York, N. Y.

Dr. F. Raymond Keating, Consultant in Clinical Medicine, Mayo Clinic, Rochester, Minnesota.

Dr. B. J. Kennedy, Assistant Professor of Medicine, University of Minnesota Medical School, Minneapolis, Minnesota.

Dr. Irvine McQuarrie, Professor of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota.

Dr. Robert L. Noble, Professor and Head, Department of Physiology, The Collip Medical Research Laboratory, The University of Western Ontario, London, Ontario.

Dr. Edward C. Reifenstein, Jr., Director, Oklahoma Medical Research Institute and Hospital; Professor of Research Medicine, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma.

Dr. Willard O. Thompson, Clinical Professor of Medicine, University of Illinois College of Medicine, Chicago, Illinois.

Dr. Sidney C. Werner, Associate in Medicine, College of Physicians and Surgeons, Columbia University, New York, N. Y.

Dr. Robert H. Williams, Professor and Chairman, Department of Medicine, University of Washington School of Medicine, Seattle, Washington.

PROGRAM

Monday, September 28, 1953

8:00– 8:45 A.M. *Registration:* Dormitory Desk, Center for Continuation Study
8:45– 9:00 A.M. Introductory Remarks—*Dr. Reifenstein, Dr. Flink*

GENERAL ENDOCRINOLOGY AND METABOLISM

Chairman of Morning Session: *Dr. Reifenstein*

9:00– 9:45 A.M. Lecture 1. General Principles of Endocrinology—*Dr. Reifenstein*
9:45–10:15 A.M. Lecture 2. Somatic and Sexual Growth, Development and Senescence—*Dr. Noble*
10:15–10:45 A.M. *Intermission*—Coffee—Center Lounge
10:45–11:15 A.M. Lecture 3. Abuse of Hormones, *Dr. Williams*
11:15–12:00 NOON Lecture 4. Endocrine Control of Protein, Fat, Carbohydrate, Water and Electrolytes—*Dr. Noble*
12:00– 1:30 P.M. *Intermission*—Luncheon—Center Dining Room

Chairman of Afternoon Session: *Dr. Noble*

1:30– 3:15 P.M. Lecture 5. Demonstration of Tests and Assays—*Dr. Greenblatt*, Director
3:15– 3:45 P.M. *Intermission*—Coffee—Center Lounge
3:45– 4:30 P.M. Lecture 6. Uses and Limitations of Laboratory Tests in Certain Endocrine Conditions—*Dr. Evans*
4:30– 5:00 P.M. Lecture 7. Clinical and Metabolic Studies on Periodic Paralysis—*Dr. McQuarrie*
5:00– 5:30 P.M. QUESTION AND ANSWER PANEL DISCUSSION—*Dr. Evans*, Moderator
6:30 P.M. GET-ACQUAINTED PARTY

Tuesday, September 29, 1953

ADRENAL CORTEX

Chairman of Morning Session: *Dr. Werner*

8:00– 8:30 A.M. Lecture 8. Chemistry, Physiology and Laboratory Tests—*Dr. Flink*
8:30– 9:15 A.M. Lecture 9. Cushing's Syndrome—*Dr. Greenblatt*
9:15– 9:30 A.M. Lecture 10. Cushing's Syndrome in Children—*Dr. McQuarrie*
9:30–10:15 A.M. Lecture 11. Cortisone and ACTH in Arthritis, Collagen Diseases, and so forth—*Dr. Jailer*
10:15–10:45 A.M. *Intermission*—Coffee—Center Lounge
10:45–11:30 A.M. Lecture 12. Adrenal Cortical Insufficiency—*Dr. Jailer*
11:30–12:00 NOON QUESTION AND ANSWER PANEL DISCUSSION—*Dr. Flink*, Moderator
12:00– 1:30 P.M. *Intermission*—Luncheon—Center Dining Room

Chairman of Afternoon Session: *Dr. Jailer*

1:30– 2:15 P.M. Lecture 13. Adrenogenital and Related Syndromes—*Dr. Werner*
2:15– 2:45 P.M. Lecture 14. Differential Diagnosis between Adrenal Neoplasia and Hyperplasia, *Dr. Jailer*
2:45– 3:30 P.M. Lecture 15. Metabolic Consequences of Stress—*Dr. Werner*
3:30– 4:00 P.M. *Intermission*—Coffee—Center Lounge
4:00– 4:30 P.M. QUESTION AND ANSWER PANEL DISCUSSION—*Dr. Greenblatt*, Moderator
4:30– 5:30 P.M. CASE PRESENTATIONS

Wednesday, September 30, 1953

THYROID

Chairman of Morning Session: *Dr. Keating*

8:00– 8:30 A.M. Lecture 16. Chemistry, Physiology and Laboratory Tests—*Dr. Albert*
8:30– 9:15 A.M. Lecture 17. Hyperthyroidism—*Dr. Williams*
9:15– 9:30 A.M. Lecture 18. Exophthalmos—*Dr. Albert*
9:30–10:15 A.M. Lecture 19. Hypothyroidism—*Dr. Keating*
10:15–10:45 A.M. *Intermission*—Coffee—Center Lounge
10:45–11:10 A.M. Lecture 20. Thyroiditis and Thyroid Cancer—*Dr. Werner*
11:10–11:30 A.M. Lecture 21. Diagnostic Uses of Radioactive Iodine—*Dr. Keating*
11:30–12:00 NOON QUESTION AND ANSWER PANEL DISCUSSION—*Dr. Williams*, Moderator
12:00– 1:30 P.M. *Intermission*—Luncheon—Center Dining Room

ANTERIOR PITUITARY

Chairman of Afternoon Session: *Dr. Thompson*

1:30– 2:00 P.M. Lecture 22. Chemistry, Physiology and Laboratory Tests—*Dr. Albert*
2:00– 2:30 P.M. Lecture 23. Acromegaly and Gigantism—*Dr. Williams*
2:30– 3:00 P.M. Lecture 24. Hypopituitarism—*Dr. Hurxthal*
3:00– 3:30 P.M. Lecture 25. Dwarfism—*Dr. Thompson*
3:30– 4:00 P.M. *Intermission*—Coffee—Center Lounge
4:00– 4:30 P.M. QUESTION AND ANSWER PANEL DISCUSSION—*Dr. Albert*, Moderator
4:30– 5:30 P.M. CASE PRESENTATIONS

Thursday, October 1, 1953

TESTIS

Chairman of Morning Session: *Dr. Engle*

8:00– 8:30 A.M. Lecture 26. Chemistry, Physiology and Laboratory Tests—*Dr. Engle*
8:30– 9:15 A.M. Lecture 27. Hypogonadism in the Male—*Dr. Thompson*
9:15– 9:30 A.M. Lecture 28. Cryptorchidism—*Dr. Hurxthal*
9:30–10:15 A.M. Lecture 29. Endocrine Management of Cancer of the Prostate—*Dr. Kennedy*
10:15–10:45 A.M. *Intermission*—Coffee—Center Lounge
10:45–11:00 A.M. Lecture 30. Testicular Tumors—*Dr. Thompson*
11:00–11:30 A.M. Lecture 31. Gynecomastia—*Dr. Kennedy*
11:30–12:00 NOON QUESTION AND ANSWER PANEL DISCUSSION—*Dr. Hurxthal,* Moderator
12:00– 1:30 P.M. *Intermission*—Luncheon—Center Dining Room

PANCREAS

Chairman of Afternoon Session: *Dr. Evans*

1:30– 2:00 P.M. Lecture 32. Chemistry, Physiology and Laboratory Tests—*Dr. Evans*
2:00– 2:50 P.M. Lecture 33. Diabetes Mellitus—*Dr. Hurxthal*
2:50– 3:10 P.M. Lecture 34. Sensitivity and Resistance to Insulin—*Dr. Evans*
3:10– 3:30 P.M. Lecture 35. Hypoglycemia and Hyperinsulinism—*Dr. McQuarrie*
3:30– 4:00 P.M. *Intermission*—Coffee—Center Lounge
4:00– 4:30 P.M. QUESTION AND ANSWER PANEL DISCUSSION—*Dr. McQuarrie,* Moderator
4:30– 5:30 P.M. CASE PRESENTATIONS

Friday, October 2, 1953

OVARY

Chairman of Morning Session: *Dr. Hurxthal*

8:00– 8:30 A.M. Lecture 36. Chemistry, Physiology and Laboratory Tests—*Dr. Engle*
8:30– 9:30 A.M. Lecture 37. Hypogonadism in the Female—*Dr. Hurxthal*
9:30–10:15 A.M. Lecture 38. Functional Ovarian Disorders and Tumors—*Dr. Engle*
10:15–10:45 A.M. *Intermission*—Coffee—Center Lounge
10:45–11:30 A.M. Lecture 39. Endocrine Management of Cancer of the Breast—*Dr. Kennedy*
11:30–12:00 NOON QUESTION AND ANSWER PANEL DISCUSSION—*Dr. Kennedy,* Moderator
12:00– 1:30 P.M. *Intermission*—Luncheon—Center Dining Room

Chairman of Afternoon Session: *Dr. Kennedy*

1:30– 2:15 P.M. Lecture 40. Post Menopausal Osteoporosis—*Dr. Reifenstein*
2:15– 3:00 P.M. Lecture 41. Sterility in Males and Females—*Dr. Engle*
3:00– 3:30 P.M. Lecture 42. Diabetes Insipidus—*Dr. Flink*
3:30– 4:00 P.M. *Intermission*—Coffee—Center Lounge
4:00– 4:30 P.M. QUESTION AND ANSWER PANEL DISCUSSION—*Dr. Engle,* Moderator
4:30– 5:30 P.M. CASE PRESENTATIONS

Saturday, October 3, 1953

PARATHYROID

Chairman of Morning Session: *Dr. Flink*

8:00– 8:30 A.M. Lecture 43. Chemistry, Physiology and Laboratory Tests—*Dr. Reifenstein*
8:30– 9:15 A.M. Lecture 44. Hyperparathyroidism—*Dr. Keating*
9:15–10:00 A.M. Lecture 45. Hypoparathyroidism—*Dr. Flink*
10:00–10:30 A.M. *Intermission*—Coffee—Center Lounge
10:30–11:00 A.M. QUESTION AND ANSWER PANEL DISCUSSION—*Dr. Reifenstein,* Moderator
11:00–11:45 A.M. CASE PRESENTATIONS
11:45–12:00 NOON Closing Remarks—*Dr. Reifenstein, Dr. Flink*

THE NEW YORK ACADEMY OF MEDICINE

The New York Academy of Medicine is holding its Twenty-Sixth Annual Graduate Fortnight this year from October 19 to 30, inclusive. The subject will be *Disorders of the Blood and the Blood-Forming Organs.*

The program will consist of twenty evening lectures, six morning panel meetings and afternoon clinics presented by twenty participating hospitals in the metropolitan area, as well as a scientific exhibit.

A program and registration card will be sent to Non-Fellows of the Academy upon receipt of a check, payable to The New York Academy of Medicine, for $10.00 for the entire program, or $5.00 for the second week.